Time-restricted feeding protects against cisplatin-induced acute kidney injury in mice

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Background: Time-restricted feeding (TRF), devoid of calorie restriction, is acknowledged for promoting metabolic health and mitigating various chronic metabolic diseases. While TRF exhibits widespread benefits across multiple tissues, there is limited exploration into its impact on kidney function. In this study, our aim was to investigate the potential ameliorative effects of TRF on kidney damage in a mouse model of cisplatin-induced acute kidney injury (AKI).

Methods: Cisplatin-induced AKI was induced through intraperitoneal injection of cisplatin into C57BL/6 male mice. Mice undergoing TRF were provided unrestricted access to standard chow daily but were confined to an 8-hour feeding window during the dark cycle for 2 weeks before cisplatin injection. The mice were categorized into four groups: control, TRF, cisplatin, and TRF + cisplatin.

Results: The tubular damage score and serum creatinine levels were significantly lower in the TRF + cisplatin group compared to the cisplatin group. The TRF + cisplatin group exhibited reduced expression of phosphorylated nuclear factor kappa B, inflammatory cytokines, and F4/80-positive macrophages compared to the cisplatin group. Furthermore, oxidative stress markers for DNA, protein, and lipid were markedly decreased in the TRF + cisplatin group compared to the cisplatin group. TUNEL-positive tubular cells, cleaved caspase-3 expression, and the Bax/Bcl-2 ratio in the TRF + cisplatin group were lower than those in the cisplatin group.

Conclusion: TRF, without calorie restriction, effectively mitigated kidney damage by suppressing inflammatory reactions, oxidative stress, and tubular apoptosis in a mouse model of cisplatin-induced AKI. TRF holds promise as a novel dietary intervention for preventing cisplatin-induced AKI.

Keywords: Acute kidney injury, Apoptosis, Cisplatin, Inflammation, Intermittent fasting, Oxidative stress

Introduction

Acute kidney injury (AKI) is a renal disorder characterized by a rapid decline in kidney function, and its high morbidity and mortality make it a global health problem [1,2]. Among the various causes of AKI, nephrotoxic drugs are responsible for approximately 20% of AKI cases in both hospital and outpatient settings [3]. One of these nephrotoxic agents is cisplatin, an inorganic platinum-based chemotherapeutic agent widely used in the treatment of various solid cancers [4]. However, its use is limited by side effects in normal tissues and organs, and nephrotoxicity is the principal limitation of cisplatin cancer therapy, affecting approximately one-third of patients receiving cisplatin...
therapy [4,5]. Despite significant progress in studying the pathophysiology of cisplatin-induced AKI over the past decades, there is still no effective prophylaxis against it. Therefore, the development of new prophylactic strategies for cisplatin-induced AKI holds great clinical significance.

Calorie restriction, a reduction in caloric intake without malnutrition, has consistently been found to produce reductions in body weight and extend a healthy lifespan across a variety of species [6]. Furthermore, calorie restriction promotes metabolic health and ameliorates chronic metabolic diseases, such as type 2 diabetes mellitus and cardiovascular disorders [7]. Regarding kidney disease, calorie restriction is known to trigger an adaptive defense program to increase resistance to stress and ameliorate renal ischemia-reperfusion injury (IRI) in experimental animals [8–10]. Thus, efforts have been made to pave the way for an alternative, non-pharmacological, diet-based approach to prevent organ injury in both animals and humans [7]. However, although several studies highlight the benefits of calorie restriction in humans, findings from obesity intervention trials over the past decades indicate that the vast majority of humans have significant difficulty sustaining daily calorie restriction for long periods of time [6].

Time-restricted feeding (TRF), a novel dietary intervention where animals have access to food for a defined period of the day, has gained popularity as an alternative to calorie restriction and has shown promise in delivering similar benefits in terms of weight loss and cardiometabolic health [11,12]. TRF does not restrict caloric consumption during the time-restricted window, which is considered an advantage in terms of improving adherence, as no calorie count is needed [13]. Currently, although preclinical and clinical trials have shown that TRF has broad-spectrum benefits for many health conditions such as obesity, diabetes mellitus, cardiovascular disease, cancers, and neurological disorders [12], few studies to date have focused on its effect on the kidney. In the current study, we investigate whether TRF protects against renal injury in a mouse model of cisplatin-induced AKI.

Methods

Animals

All animal experiments were approved by the Institutional Animal Care and Use Committee of Pusan National University (PNU-IACUC, 2021-032) and conducted according to the Guidelines for the Care and Use of Experimental Animals endorsed by the Korean Society of Experimental Animals. Male C57BL/6 mice (8 weeks old) were purchased from the Koatech Technology Corporation (Seoul, Korea) and housed in a specific pathogen-free facility at 22 ± 1 °C, 55% ± 5% humidity under a 12-hour light/dark cycle. 

Animal experimental design

After acclimatization for at least 1 week, the mice were randomly divided into four groups as follows (n = 6/group) (Fig. 1A): (a) sham: ad libitum food and vehicle injection, (b) TRF: TRF and vehicle injection, (c) cisplatin: ad libitum food and cisplatin injection, (d) TRF + cisplatin: TRF and cisplatin injection. Mice with ad libitum access had 24-hour availability of food, while the mice on TRF were provided with food for 8 hours daily, starting 3 hours after the beginning of the dark cycles (from 9:00 p.m. to 5:00 a.m.), for a total of 2 weeks. After 2-week of either ad libitum feeding or TRF, the cisplatin (15 mg/kg in 0.9% saline; Sigma-Aldrich) was injected intraperitoneally into the cisplatin group and the TRF + cisplatin group. An equal volume of the vehicle (0.9% saline) was injected intraperitoneally into the sham group and the TRF group. All mice were provided with ad libitum food for 72 hours after cisplatin or vehicle injection. All mice were provided with ad libitum food for 72 hours after cisplatin or vehicle injection.

Blood measurements

Blood glucose and β-hydroxybutyrate (β-HB) levels were measured using a blood glucose and ketone monitoring meter (FreeStyle Optium, Neo; Abbott) in whole blood obtained from the tail vein of mice after 2 weeks of either TRF or ad libitum feeding, immediately before cisplatin administration. Serum creatinine levels were assayed using enzyme-linked immunosorbent assays (#80; Crystal Chem) at the time of mice sacrifice.

Tissue preparation

The mice were euthanized 72 hours after cisplatin administration. The kidneys were perfused with cold (4 °C)
Figure 1. Effect of TRF on metabolic parameters. (A) Experimental design. TRF were provided with food for 8 hours daily for a total of 2 weeks. (B) Food intake. Mice enrolled in TRF for 2 weeks consumed a similar amount of food as the ad libitum control group. (C) Body weight. TRF did not lead to any bodyweight reduction. (D) β-hydroxybutyrate (β-HB) level. TRF-treated mice showed increased levels of β-HB. (E) Glucose level. There was no difference in blood glucose levels between TRF-treated and ad libitum-treated mice. Cis, cisplatin; TRF, time-restricted feeding. Data are expressed as mean ± standard deviation (n = 6/group; *p < 0.05 compared with sham and cisplatin group).

phosphate-buffered saline and immediately resected. One kidney was stored at ~80 °C for protein and messenger RNA (mRNA) analyses. The remaining kidney was fixed in 10% neutralized formalin at room temperature and embedded in paraffin for histological and immunohistochemical analyses.
Histological analyses

The paraffin-embedded kidney samples were cut into 4-μm sections and stained with hematoxylin and eosin (H&E), as described previously [14]. The kidney sections underwent deparaffinization, rehydration in distilled water, staining with Mayer’s hematoxylin for 1 minute, and washing with 4–5 changes of tap water. They were subsequently rinsed with three changes of distilled water, counterstained in alcoholic eosin for 1 minute, and dehydrated through three changes of 95% ethanol and two changes of 100% ethanol. All stained kidney tissues were digitally scanned using a ZEISS Axioscan 7 (Carl Zeiss). Images were captured at 20× magnification using ZEN Lite microscope software (Carl Zeiss), and 10 high power fields (HPFs) were randomly selected. The degree of tubular injury was evaluated by a pathologist in a blinded manner, based on criteria such as tubular dilatation, loss of brush border, vacuolization, epithelial cell shedding, and denuded tubular basement membrane. The scoring scale was as follows: 0, normal; 1, <10%; 2, 10%–25%; 3, 25%–50%; 4, 50%–75%; and 5, 75%–100% [15].

Immunohistochemical analyses

Immunohistochemical analyses were performed on 3-μm paraffin-embedded kidney sections, as described previously [14]. In summary, the sections were deparaffinized and rehydrated in an ethanol series. After microwave-based antigen retrieval, the sections were blocked with normal horse serum (Vector Laboratories) and left to incubate overnight with primary antibodies at 4 °C. Subsequently, the sections were treated with secondary antibodies (ImmPRESS HRP reagent kit; Vector Laboratories) for 30 minutes at 37 °C. Finally, the slides were developed using 3,3-diaminobenzidine tetrahydrochloride (Vector Laboratories) and counterstained with hematoxylin. Primary antibodies used were anti-F4/80 (#ab111101; Abcam) and anti-phosphorylated nuclear factor kappa B (p-NF-κB) p65 (#sc-136548; Santa Cruz Biotechnology), anti-8-hydroxy-2′-deoxyguanosine (8-OHdG) (JalCA), anti-4-hydroxy-2-nonenal (4-HNE) (JalCA), and anti-nitrotyrosine (#ab125106; Abcam). The stained kidney tissues were captured using a ZEISS Axioscan 7. The images were obtained using the ZEN Lite microscope software, and 10 HPFs were selected randomly for assessment. The image analysis excluded glomeruli, large vessels, and perivascular and subcapsular regions. The average counts of F4/80- and p-NF-κB p65-positive cells per HPF were evaluated in a blinded manner. The staining of 8-OHdG, 4-HNE, and nitrotyrosine was semi-quantitatively graded in a blinded manner using the following scale: 0 for no stained area; 1 for 1%–25% stained area; 2 for 26%–50% stained area; 3 for 51%–75% stained area; and 4 for >75% stained area.

Western blotting

Western blotting was performed as described previously [14]. Briefly, proteins were extracted from kidneys with a protein extraction solution (PRO-PREP; iNtRON Biotechnology). Protein concentrations were quantified by the Bradford method (Bio-Rad Protein Assay; Bio-Rad Laboratories Inc.). Proteins were separated by electrophoresis on 12% sodium dodecyl sulfate-polyacrylamide gels and transferred onto nitrocellulose membranes (Hybond ECL; Amersham Pharmacia Biotech Inc.). The membranes were incubated for 2 hours at room temperature with a solution containing 5% (w/v) nonfat dried milk in Tris-buffered saline (10 mM Tris/HCl, pH 8.0, and 150 mM NaCl) supplemented with 0.05% Tween-20. Following this, the membranes were probed using the following specific primary antibodies: anti-Bcl-2 (#ABP50759; Abbkine), anti-Bax (#ABP50752; Abbkine), anti-caspase-3 (#ab13847; Abcam), and cleaved caspase-3 (#9664; Cell Signaling Technology). The secondary antibodies used were anti-rabbit or anti-mouse immunoglobulin G antibodies (#ADI-SAB-300-J, #ADI-SAB-100-J; Enzo Life Science). Immunoreactive bands were visualized using enhanced chemiluminescence (Pierce ECL Western blotting substrate; Thermo Scientific). The protein levels were quantified with ImageJ software (National Institutes of Health). To determine protein expression levels, the relative expression of the target protein was normalized to β-actin (Cell Signaling Technology).

TUNEL staining

Tubular apoptosis was assessed using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining. Kidney paraffin sections, 3 μm-thick, were de-
paraffinized in xylene and rehydrated through a series of ethanol solutions. The TUNEL assay was conducted using the TUNEL Apoptosis Detection Kit (#G3250; Promega), following the manufacturer’s protocol. Nuclei were counterstained with DAPI. Stained kidney tissues were imaged using a ZEISS Axioscan 7. Images were captured using ZEN Lite microscope software at ×20 magnification. TUNEL-positive cell number was quantified in 10 randomly selected HPFs and averaged by an observer blinded to the samples.

Quantitative reverse-transcription polymerase chain reaction analysis

Quantitative reverse-transcription polymerase chain reaction analysis was conducted as previously described [14]. The GAPDH (glyceraldehyde 3-phosphate dehydrogenase) was employed as the housekeeping internal control and was quantified simultaneously with the target gene (tumor necrosis factor alpha [TNF-α], interleukin [IL]-6, IL-1α). All products were confirmed through melting curve analysis (95 °C for 15 seconds, 60 °C for 45 seconds, and 72 °C for 1 minute). Normalization and fold-change values for each gene were calculated by the 2^ΔΔCT method. The primer sequences used are listed in Table 1.

Statistical analysis

Data were presented as the mean ± standard deviation. Analysis of the data was conducted using the Mann-Whitney U test or Kruskal-Wallis test, as deemed appropriate. All statistical analyses were carried out using IBM SPSS version 21.0 (IBM Corp.). A p-value of <0.05 was considered statistically significant.

Table 1. Sequences of the real-time PCR primers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward (5′-3′)</th>
<th>Reverse (3′-5′)</th>
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<tbody>
<tr>
<td>GAPDH (mouse)</td>
<td>CATCACTGCCACCCAGAAGACTG</td>
<td>TGCCAGTGAAGTCTCCGTGTTCA</td>
</tr>
<tr>
<td>TNF-α (mouse)</td>
<td>GGTGCCATATGCTCTAGCGTCTTT</td>
<td>GCCATAGAATGTAGAGAGGAGGAG</td>
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<tr>
<td>IL-6 (mouse)</td>
<td>TACACTTCACAAGTCGGAGGC</td>
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<tr>
<td>IL-1α (mouse)</td>
<td>ACGGCTAGTTTTCCAGAGTGC</td>
<td>CACTCTGGTATAGTGGTAGTGGTGC</td>
</tr>
</tbody>
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GAPDH, glyceraldehyde 3-phosphate dehydrogenase; IL, interleukin; PCR, polymerase chain reaction; TNF, tumor necrosis factor.

Results

Effect of time-restricted feeding on metabolic parameter

Mice enrolled in TRF for 2 weeks consumed a similar amount of food as the ad libitum control group, confirming that there is no caloric restriction during TRF (Fig. 1B). TRF also did not lead to any body weight reduction (Fig. 1C). Additionally, TRF-treated mice showed increased levels of β-HB, indicating that the TRF regimen induced a state of ketosis (Fig. 1D). There was no difference in blood glucose levels between TRF-treated and ad libitum-treated mice (Fig. 1E).

Time-restricted feeding attenuates renal dysfunction and pathological damage in cisplatin-induced acute kidney injury

The serum creatinine levels of the cisplatin group were higher than those of the sham group 72 hours after cisplatin administration. However, the serum creatinine levels of the TRF + cisplatin group were lower than those of the cisplatin group, indicating that the TRF regimen attenuated renal dysfunction in a mouse model of cisplatin-induced AKI (Fig. 2A). Cisplatin is known to induce tubular injury in humans and rodents [4]. Therefore, we performed a histological assessment of each group using H&E-stained kidney sections. The cisplatin group exhibited prominent histopathological alteration, such as tubular dilation, cast formation, loss of brush border, vacuolization, and epithelial cell shedding than the sham group. However, all these injuries were significantly mitigated in the TRF + cisplatin group (Fig. 2B, C).

Table 1. Sequences of the real-time PCR primers
Time-restricted feeding attenuates the inflammatory reaction in cisplatin-induced acute kidney injury

Since inflammatory reactions play a pivotal role in cisplatin-induced AKI [16], we investigated the mRNA expression of pro-inflammatory cytokines such as TNF-α, IL-1α and IL-6, in kidney tissues. The TNF-α, IL-1α, and IL-6 mRNA expression levels in the cisplatin group were significantly higher than those in the sham group. However, TNF-α, IL-1α, and IL-6 mRNA expression levels in the TRF + cisplatin group were significantly lower than those in the cisplatin group (Fig. 3A). Moreover, the number of F4/80-stained cells, a pan-macrophage marker, in the interstitial space of the cisplatin group was higher than that in the sham group. However, the number of F4/80-stained cells in the TRF + cisplatin group was significantly lower than that in the cisplatin group (Fig. 3B).

NF-κB is an important nuclear transcript factor that induces the expression of various pro-inflammatory cytokines [17]. Therefore, we investigated NF-κB expression during inflammation in cisplatin-induced AKI. Immunohistochemical analyses showed that p-NF-κB p65 expression in the cisplatin group was higher than that in the sham group. However, p-NF-κB p65 expression in the TRF
Figure 3. Effect of TRF on inflammatory reaction in cisplatin (Cis)-induced acute kidney injury. (A) The tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, and IL-1α messenger RNA (mRNA) levels. The TNF-α, IL-6 mRNA, and IL-1α expression in the TRF + Cis group were significantly lower than those in the Cis group. (B) Representative images of immunohistochemical analysis for F4/80. The F4/80-stained area in the TRF + Cis group was significantly decreased compared to that in the Cis group. (C) Representative images of immunohistochemical analysis for phosphorylated nuclear factor kappa B (p-NF-κB) p65. A p-NF-κB p65 expression in the TRF + Cis group was lower than that in the Cis group.

HPF, high power field; TRF, time-restricted feeding.

Data are expressed as mean ± standard deviation (n = 6/group; *p < 0.05 compared with sham and TRF group; #p < 0.05 compared with cisplatin group).
cisplatin group was lower than that in the cisplatin group (Fig. 3C).

Time-restricted feeding ameliorates oxidative stress in cisplatin-induced acute kidney injury

Oxidative stress has been recognized as an important factor that contributes to cisplatin-induced AKI [4]. To evaluate whether TRF suppresses cisplatin-induced AKI, the kidney section was stained with an antibody against antibodies against 8-OHdG (a marker of DNA oxidation), 4-HNE (a marker of lipid oxidation), and nitrotyrosine (a marker of protein oxidation). We found that expression levels of nitrotyrosine, 8-OHdG, and 4-HNE in the cisplatin group were markedly increased compared to that in the sham group. However, these changes were significantly mitigated by TRF (Fig. 4).

Time-restricted feeding ameliorates tubular apoptosis in cisplatin-induced acute kidney injury

In addition to tubular necrosis, tubular apoptosis plays an important role in cisplatin-induced AKI [18]. Thus, we investigated whether TRF ameliorated tubular apoptosis in cisplatin-induced AKI. Tubular cell apoptosis was analyzed by TUNEL staining. The number of apoptotic tubular cells in the cisplatin group was considerably higher than that in the sham group. However, the number of apoptotic cells in the TRF + cisplatin group was lower than that in the cisplatin group (Fig. 5A).

Caspa-3 is the major caspase detected in apoptotic cells [19]. Bcl-2 is an anti-apoptotic protein, whereas Bax is a pro-apoptotic protein [19]. Therefore, we examined the expression of caspase-3, Bax, and Bcl-2 to investigate the effect of TRF on renal tubular apoptosis in cisplatin-induced AKI. Western blotting revealed that the cleaved caspase-3 (the activated form of caspase-3) expression and Bax/Bcl-2 expression ratio in the cisplatin group was significantly higher than that in the sham group. However, cleaved caspase-3 expression and Bax/Bcl-2 expression ratio in the TRF + cisplatin group were lower than those in the cisplatin group (Fig. 5B).

Discussion

The pathophysiology of cisplatin-induced AKI is complex. Although the precise mechanisms are not fully elucidated, accumulating evidence suggests the involvement of inflammatory responses, apoptosis of tubular cells, and oxidative stress in the pathophysiology of the disease [4,5]. Among these factors, a robust inflammatory response triggered by cisplatin plays a significant role in the functional and structural deterioration of the kidneys [5]. Previous studies have reported that genetic or pharmacological suppression of pro-inflammatory cytokines can alleviate cisplatin-induced AKI [5]. Moreover, oxidative stress emerges as a pivotal pathogenic element in cisplatin-induced AKI. The increased production of reactive oxygen species during cisplatin treatment activates multiple signaling cascades, leading to damage and demise of renal tubular epithelial cells [20]. Lastly, over the past decades, researchers have paid substantial attention to the apoptosis of renal tubular epithelial cells as a crucial aspect of cisplatin-induced AKI mechanisms. Several studies have indicated that inhibiting tubular cell apoptosis mitigates the impact of cisplatin-induced AKI [4,5,21]. In the context of these well-established mechanisms of cisplatin-induced AKI, our present study highlights that TRF protects against cisplatin-induced AKI by suppressing inflammation, tubular apoptosis, and oxidative stress. To the best of our knowledge, our study is the first to explore the beneficial effects of TRF on cisplatin-induced AKI.

Many studies have explored the benefits of TRF on health and disease [12,22,23]. While some attribute the positive outcomes of TRF to a reduction in overall food intake, others have demonstrated improvements in cardiometabolic markers and protection against pathological conditions, even with the implementation of isocaloric diets [23]. In our research, mice in the TRF groups exhibited similar body weights and consumed comparable amounts of food compared with the sham group, indicating the absence of calorie restriction. This suggests that the favorable effects of TRF on cisplatin-induced AKI in our study were not a result of reduced caloric intake.

One of the key findings in our study is the notable suppression of the inflammatory response by TRF in a mouse model of cisplatin-induced AKI. Although the specific mechanisms governing the anti-inflammatory effect of TRF
Figure 4. Effect of TRF on oxidative stress in cisplatin (Cis)-induced acute kidney injury. Representative images of immunohistochemical analysis for 8-OHdG (A), 4-HNE (B), and nitrotyrosine (C). The expression of 8-OHdG, 4-HNE, and nitrotyrosine in the TRF + Cis group was markedly decreased compared to that in the Cis group.

HPF, high power field; TRF, time-restricted feeding; 4-HNE, 4-hydroxy-2-nonenal; 8-OHdG, 8-hydroxy-2'-deoxyguanosine.

Data are expressed as mean ± standard deviation (n = 6/group; *p < 0.05 compared with sham and TRF group; #p < 0.05 compared with cisplatin group).
on cisplatin-induced AKI were not delineated in this investigation, TRF is recognized for its intrinsic anti-inflammatory properties. In a meta-analysis encompassing 25 human studies, TRF is proposed as an effective approach to diminish TNF-α and leptin levels in the general adult population [24]. Previous research, both in humans and animals, has demonstrated that TRF reduces inflammatory responses in chronic inflammatory conditions, including cardiovascular disease, diabetes mellitus, neurodegenerative disorders, and arthritis [22]. Concerning kidney disease, TRF is reported to decrease renal innate immune cells in a mouse model of hypertension [23].

Despite these findings, the precise mechanism by which TRF mitigates inflammation in our study remains unclear. One potential mechanism is TRF-induced ketosis. We observed elevated levels of β-HB in TRF-treated mice, indicating that the TRF regimen induced a state of ketosis. As the most abundant ketone body in mammals, β-HB is a metabolic byproduct synthesized during fat breakdown in the liver, serving as an alternative energy source in response to carbohydrate or glucose depletion [25]. Beyond its role as an energy substrate, β-HB emerges as a crucial signaling molecule, with a distinct capacity to modulate inflammatory responses [25]. Facilitated by monocarboxylic acid transporters, β-HB reaches cells, contributing to energy generation and enhancing anti-inflammatory signaling [26]. Its anti-inflammatory action extends to the modulation of peroxisome proliferator-activated receptor-gamma co-

Figure 5. Effect of TRF on tubular apoptosis in cisplatin (Cis)-induced acute kidney injury. (A) Representative image of TUNEL staining. The number of apoptotic cells in the TRF + Cis group was lower than that in the Cis group. (B) Representative images of a western blot for cleaved caspase-3, Bax, and Bcl-2. The expression of cleaved caspase-3 (activated caspase-3) and the Bax/Bcl-2 expression ratio in the TRF + Cis group were lower than that in the Cis group. TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; TRF, time-restricted feeding. Data are expressed as mean ± standard deviation (n = 6/group; *p < 0.05 compared with sham and TRF group; #p < 0.05 compared with cisplatin group).
activator-1 alpha (PGC-1α) and forkhead box O1 (FOXO1) [27]. In a previous study [28], we examined the impact of pretreatment with exogenous β-HB on inflammation in a mouse model of cisplatin-induced AKI. Our findings indicated that β-HB pretreatment significantly reduced the expression of phosphorylated NF-κB, inflammatory cytokines, and macrophage infiltration. These results suggest that β-HB may play a crucial role in the anti-inflammatory effects of TRF. We posit that further investigations are necessary to elucidate mechanisms other than β-HB that contribute to the anti-inflammatory effect of TRF in cisplatin-induced AKI.

Our study uncovered the inhibitory potential of TRF against oxidative stress in a mouse model of cisplatin-induced AKI. While the precise mechanism underlying the anti-oxidative effect of TRF in cisplatin-induced AKI was not delineated in the present study, accumulating evidence suggests that TRF possesses anti-oxidative properties. A systemic review of randomized controlled studies indicates that TRF has the potential to decrease circulating markers of oxidative stress [29]. Previous animal and human studies have also suggested that TRF decreased oxidative stress markers in chronic disease (obesity, asthma, type 2 diabetes mellitus, cardiovascular disease, and cancer) [12,13,22]. Regarding kidney disease, TRF ameliorated kidney damage by increasing the antioxidant protection and preventing oxidative DNA damage in a mouse model of IRI [7]. Currently, the molecular mechanisms are unclear, but it is possible nuclear factor erythroid 2-related factor 2 (NRF2) and PGC-1a, both redox-sensitive transcriptional regulators are involved in the beneficial effects of TRF on oxidative stress [7]. Moreover, β-HB induced by TRF is also reported to activate FOXO1 and NRF2, controlling cytoprotective genes involved in the oxidative stress response [30]. Moreover, in our previous study [28], we examined the impact of exogenous β-HB on oxidative stress in a mouse model of cisplatin-induced AKI. Our observations revealed that exogenous β-HB decreased oxidative stress markers for DNA, protein, and lipid. These results suggest that β-HB plays a significant role in the anti-oxidative effect of TRF, complementing its anti-inflammatory properties. Further studies are warranted to unveil the mechanisms behind the anti-oxidative effect of TRF in cisplatin-induced AKI.

The present study also demonstrated that TRF suppressed tubular apoptosis in a mouse model of cisplatin-induced AKI. Our findings align with previous research illustrating the anti-apoptotic properties of TRF in diverse animal models. Specifically, TRF has been documented to diminish apoptosis in a mouse model of hepatic IRI [31] and to mitigate apoptosis through the modulation of autophagy in a doxorubicin-induced cardiotoxicity model of albino rats [32]. In the context of kidney disease, preoperative TRF administration demonstrated protective effects against renal IRI by inhibiting apoptosis in a mouse model of aortic aneurysm [33]. Additionally, TRF exhibited the capacity to suppress tubular apoptosis in a rat model of glycerol-induced AKI [34,35]. Despite these compelling outcomes, the exact mechanism underlying TRF’s attenuation

Figure 6. Schematic illustration of the protective effect of TRF against renal damage in a mouse model of cisplatin-induced acute kidney injury. TRF mitigated kidney damage by suppressing the inflammatory reaction, oxidative stress, and tubular apoptosis in a mouse model of cisplatin-induced acute kidney injury. IL, interleukin; NF-κB, nuclear factor kappa B; TNF-α, tumor necrosis factor alpha; TRF, time-restricted feeding; 8-OHdG, 8-hydroxy-2’-deoxyguanosine; 4-HNE, 4-hydroxy-2-nonenal.
of tubular apoptosis in a mouse model of cisplatin-induced AKI remains unclear. One plausible hypothesis is that the reduction in inflammatory reactions and oxidative stress induced by TRF contributes to the attenuation of tubular apoptosis. This is because cisplatin-induced tubular cell apoptosis is reported to be aggravated by the concurrent induction of inflammation and oxidative stress caused by cisplatin. Consequently, additional investigations are warranted to elucidate the impact of TRF on tubular apoptosis in the context of cisplatin-induced AKI.

The conclusions of the present study are summarized in Fig. 6. We observed an increase in inflammatory reactions, tubular apoptosis, and oxidative stress in a mouse model of cisplatin-induced AKI. TRF ameliorated cisplatin-induced AKI by attenuating inflammation, tubular cell apoptosis, and oxidative stress. Thus, TRF may represent a new dietary intervention for the prevention of cisplatin-induced AKI. We believe that further studies are necessary to elucidate the mechanism underlying the favorable effects of TRF on inflammation, apoptosis, and oxidative stress.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Data sharing statement

The data presented in this study are available from the corresponding author upon reasonable request.

Authors’ contributions

Conceptualization: KWJ, SBL, IYK
Data curation, Formal analysis: All authors
Funding acquisition: IYK
Investigation: KWJ, YSK, SRK, IYK
Writing–original draft: KWJ, IYK
Writing–review & editing: KWJ, IYK
All authors read and approved the final manuscript.

References