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Assessing the potential protective effects of tirzepatide against renal ischaemia-reperfusion injury in male rats: inhibition of *Foxo1* expression and pyroptosis

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ABSTRACT

Renal ischaemia-reperfusion injury (RIRI) is a significant contributing factor to acute kidney injury, characterized by inflammation and oxidative stress formation that delay tissue repair. Our study has assessed the nephroprotective effects of tirzepatide on RIRI, through its effect on the forkhead box O1 gene (*Foxo1*) expression and pyroptosis in male rats. Our study included 24 Wistar rats divided to four groups (n=6 each): a sham group (without an induction of RIRI), a control group (with an induction of RIRI), a vehicle group (receiving an intraperitoneal injection of dimethyl sulfoxide at 1 mg/kg, 1 h before ischaemia), and a tirzepatide-treated group (receiving an intraperitoneal injection of tirzepatide at 1.35 mg/kg, 1 h before ischaemia). Our results revealed that tirzepatide can significantly reduce the kidney injury molecule-1 levels (p=0.027) and the levels of pyroptosis markers caspase-1 (p<0.001) and caspase-11 (p=0.005) in the studied rats' kidneys. Histopathological analysis of the latter revealed severe injury in the control group, while tirzepatide-treated rats exhibited injury scores under 40%. Additionally, the *Foxo1* expression was found to be significantly reduced as a result of the tirzepatide treatment (p=0.031). We concluded that tirzepatide may potentially relieve ischaemic renal damage through the modulation of pyroptosis and inflammatory pathways, thereby highlighting its potential as a targeted therapy for RIRI.

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1. Introduction

Renal ischaemia-reperfusion injury (RIRI) constitutes a significant aetiological factor in the onset of acute kidney injury and has been associated with morbidity and mortality[1]. The inflammatory responses and the overproduction of reactive oxygen species within the renal tissue after an RIRI can substantially impede the processes of tissue repair, thereby complicating efforts to effectively facilitate the regeneration of the RIRI-affected tissue[2].

Pyroptosis, a type of programmed cell death mediated by caspase-11 (which activates caspase-1), can trigger renal tubular cell death following renal ischaemia, leading to inflammatory cell damage[3]. The forkhead box O1 (FOXO1) gene (humans: *FOXO1*; rats: *Foxo1*) expression is up-regulated following ischaemic conditions, suggesting its critical role in triggering the expression of other genes implicated in programmed cell death as a result of RIRI[4]. At present, there are no targeted therapeutic interventions for RIRI. Nevertheless, several studies have demonstrated that the inhibition of pathways related to oxidative stress, inflammatory processes, and cellular apoptosis may facilitate the reduction of the severity of renal injury[5].

Tirzepatide is a drug used in the management of type 2 diabetes through its binding to two receptors within the body, namely the glucagon-like peptide-1 receptor (GLP-1R) and the gastric inhibitory polypeptide receptor, in order to regulate blood glucose and obesity[6]. Tirzepatide may also improve ischaemic damage *via* its anti-inflammatory effects and the reduction of cell death[7]. Therefore, the aim of our study was to assess the potential nephroprotective effect of tirzepatide against RIRI in rats through *Foxo1* expression and pyroptosis.

2. Methodology

2.1. Study design and experiments

Twenty four adult male albino Wistar rats (weighting 200–250 g) were utilized in this study. The rats were randomly allocated into four groups (n=6 each): (i) a sham group (without an induction of RIRI), (ii) a control group (with an induction of RIRI), (iii) a vehicle group (receiving an intraperitoneal injection of dimethyl sulfoxide at 1 mg/kg, 1 h before ischaemia), and (iv) a tirzepatide-treated group (receiving an intraperitoneal injection of tirzepatide at 1.35 mg/kg, 1 h before ischaemia). Anaesthesia was administered *via* an intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Ischaemia was induced through the clamping of both renal pedicles for 30 min, followed by 2 h of reperfusion.

Following reperfusion, the rats were euthanized under anaesthesia and kidney samples were collected for analysis. The right kidneys were imbedded in formalin for histological examination, while the left kidneys were kept at -80°C for the undertaking of ELISA and genetic analysis.

The undertaken histopathological assessment involved kidney samples processing, followed by staining with haematoxylin and eosin for microscopic examination. Quantitative and qualitative renal tissue damage assessment were achieved through the scoring of tissue degeneration.

Tissue homogenates from the frozen kidneys were washed, homogenized in phosphate buffered saline with protease inhibitors and Triton X-100, and they were subsequently centrifuged. Supernatants were aliquoted and frozen for the measurement of the levels of kidney injury molecule-1 (KIM-1) and of pyroptosis biomarkers (i.e., caspase-1 and caspase-11) through ELISA. In addition, total RNA extraction was undertaken for a real-time PCR analysis of the expression of *Foxo1*.

2.2. Statistical analysis

The SPSS v.26 software was utilized for the analysis of the current study's data. All values are expressed as mean ± standard deviation. One-way ANOVA (followed by a Tukey test) was used in order to assess the differences in the variables' means among the study groups, and a significance level was considered for p-values that were <0.05.

2.3. Ethical approval

Ethical approval for the present study was granted by the Central Committee for Bioethics of the University of Kufa (approval number: 20552; date: August 29, 2024).

3. Results and Discussion

The analysis of the kidney KIM-1 levels revealed sig-

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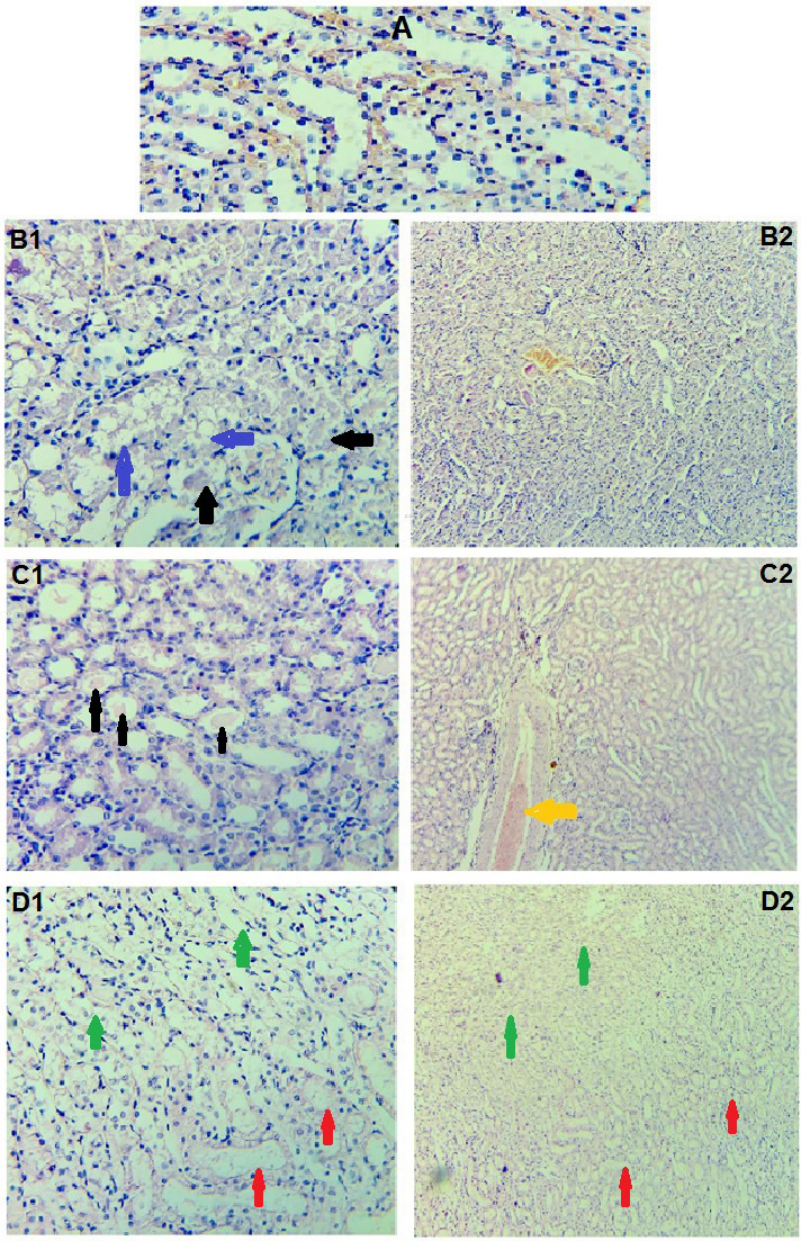

Figure 1. *Representative microphotographs from the histopathological evaluation (using haematoxylin and eosin) of kidney tissues from the four herein studied rat groups. (A): The sham group tissue samples exhibited normal glomeruli and tubules. (B1,B2): The control group tissue samples exhibited significant alterations involving the development of cytoplasmic vacuoles (blue arrows) and eosinophilic casts (black arrows). (C1,C2): The vehicle group tissue samples exhibited significant alterations involving eosinophilic casts (black arrows) and vascular congestion (orange arrow). (D1,D2): The tirzepatide-treated group tissue samples exhibited a significant improvement of the renal tissue damage when compared to that observed in the control group; damaged tubules (red arrows) and normal tubules (green arrows) are highlighted. Caption magnification: x400 (A, B1, C1, D1); x100 (B2, C2, D2).*

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nificant elevations in both the control group and the vehicle group as compared to those of the sham group (p<0.05). Importantly, the treatment with tirzepatide led to a significant reduction in the kidney KIM-1 levels (1,353.84 ± 95.37 pg/mL) compared to those of the control group (1,546.88 ± 71.72 pg/mL; p=0.027). As far as the pyroptosis markers are concerned, the kidney caspase-1 levels were found to be notably elevated in the control and vehicle groups as compared to those of the sham group (p<0.05). The administration of tirzepatide resulted in a significant decrease in the kidney caspase-1 levels (6,435.28 ± 909.65 pg/mL) when compared to those of the control group (8,402.77 ± 681.22 pg/mL; p<0.001). Finally, the kidney caspase-11 levels also exhibited a significant increase in both the control and the vehicle groups as compared to those of the sham group (p<0.05). The treatment with tirzepatide effectively reduced the kidney caspase-11 levels (2,479.15 ± 144.60 pg/mL) when compared to those of the control group (2,941.24 ± 203.60 pg/mL; p=0.005).

Histopathological examination demonstrated severe renal tissue injury in both the control and vehicle groups, with score 4 damage characterized by cytoplasmic vacuoles, eosinophilic cast, oedema, and vascular congestion. On the other hand, the tirzepatide-treated group demonstrated a significant reduction in these pathological characteristics, with injury scores indicating a less than 40% involvement of the renal tubules (Figure 1).

The kideny *Foxo1* expression levels were found to be significantly lower in both the control and vehicle groups of our study as compared to those of the sham group (p<0.001). Tirzepatide treatment resulted in a further decrease in the kidney *Foxo1* expression (0.16 ± 0.00 mg/dL) when compared to that of the control group (0.30 ± 0.03 mg/dL; p=0.031).

RIRI is a recognized challenge in the clinical setting. Our study has shown the potential protective effect of tirzepatide in mitigating ischaemic damage through its effects on pyroptosis biomarkers and the tissue levels of *Foxo1* expression. Our results revealed a significant elevation in KIM-1 and pyroptosis marker (caspase-1 and caspase-11) levels as a result of the experimentally-induced RIRI in rats. These findings are consistent with previous studies highlighting that renal ischaemia can trigger oxidative stress and inflammatory responses, which participate in the observed tubular cell death and kidney injury. In fact, the significant elevation in KIM-1 levels serves as a predictor biomarker for early tubular damage[8]. Although, to our knowledge, there are no known studies that have specifically examined the effect of tirzepatide on the herein examined parameters in rats undergoing RIRI, our data firmly suggest that tirzepatide can effectively reduce these biomarkers, thereby potentially inhibiting pyroptosis and ameliorating inflammation and renal tissue damage.

The herein presented histopathological evaluation has revealed a severe tissue injury in the ischaemic groups' tissues, characterized by the formation of cytoplasmic vacuoles, eosinophilic casts, and oedema. Previous studies have reported similar histopathological findings following ischaemic conditions in the rat kidneys[2]. The significant reduction in the recorded injury score as a result of the treatment with tirzepatide suggests that the drug can enhance tissue repair and decrease the extent of cellular damage in the rat kidneys. Alathary and Kadhim have suggested that the agonistic effects of tirzepatide on GLP-1R might be implicated in its ability to mitigate renal injury through anti-inflammatory pathways[6].

Finally, our results suggest that the rat kidney *Foxo1* expression is downregulated after renal ischaemia; this downregulation may be linked to the activation of the PI3K/AKT pathway that phosphorylates the FOXO1 transcription factor, leading to its translocation from the nucleus to the cytoplasm and the inhibition of its transcriptional activity on its own gene[9]. The herein assessed tirzepatide treatment was able to further reduce the *Foxo1* expression. As previous studies have reported that FOXO1 is involved in the regulation of various genes implicated in oxidative stress and cell death[10], the downregulation of its gene expression by tirzepatide could underscore a potential protective response.

4. Conclusion

The downregulation of the rat kidney *Foxo1* expression, along with the reduction in the pyroptosis-associated caspases, suggests potential mechanisms for the observed tirzepatide-induced nephroprotective effects.

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These findings suggest that tirzepatide could be a promising therapeutic option for mitigating RIRI.

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Conflicts of interest

None exist.

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