A short–term pretreatment with insulin and glucose efficiently protected the kidney against Ischemia-Reperfusion injury via the P-AKT-Bax-Caspase-3 signaling pathway in mice

Liwei Sun  
Zhengzhou Central Hospital Affiliated to Zhengzhou University

Hailong Bing  
Zhengzhou Central Hospital Affiliated to Zhengzhou University

Chengxi Zhang  
Zhengzhou Central Hospital Affiliated to Zhengzhou University

Lin Lin  
Zhengzhou Central Hospital Affiliated to Zhengzhou University

Hongkai Lian  
Zhengzhou Central Hospital Affiliated to Zhengzhou University

Qinjun Chu  
Zhengzhou Central Hospital Affiliated to Zhengzhou University

Xiaogao Jin  
Zhengzhou Central Hospital Affiliated to Zhengzhou University

Research Article

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Abstract

Objective

This study investigated whether pretreatment with insulin and glucose protects the kidney against ischemia-reperfusion injury (IRI).

Methods

Kidney IRI was performed in C57BL/6 mice by clamping the renal vessels for 30 min, followed by re-perfusion for 24 h. A total subcutaneous 0.1 unit of insulin along with 10% glucose in drinking water was treated on the mice for 24 h before kidney IRI. The kidney function and injuries were investigated through the determination of BUN and Cr in blood plasma, as well as the apoptosis and the expression of P-AKT, BAX, and caspase-3 in the kidneys. The role of P-AKT in insulin-treated IRI kidneys was tested using an AKT inhibitor. The effects of the pretreatment duration of insulin and glucose on IRI kidneys were investigated by expanding the treatment duration to 1, 3, and 6 days.

Results

Pretreatment with insulin and glucose protected the kidney against IRI through a decrease in Cr and BUN concentration in plasma and a reduction of kidney injuries. The protection effect was related to the signaling pathway of P-AKT-BAX-caspase-3. An AKT inhibitor partially reversed the protective effects of insulin pretreatment. The pretreatment duration for 1, 3, and 6 days had no differences in improving kidney functions and pathology.

Conclusion

A short-term pretreatment with insulin and glucose protected the kidney from IRI through the activation of p-AKT and subsequent reduction of BAX-caspase-3-induced apoptosis. The short-term pretreatment provides a practicable strategy for protecting the kidney against predictable IRI, such as major operations with high hypotension incidence.

Introduction

Acute kidney injury caused by ischemia-reperfusion injury (IRI) is a common condition in multiple clinical settings, such as septic shock, trauma, kidney transplantation, and cardiovascular surgery [1–3]. Acute kidney injury could lead to high mortality and a lengthy ICU long-time [3, 4]. Aggressive prevention of acute kidney injury remarkably reduces mortality and medical expenses for critically ill patients [5]. Insulin has been found to prevent human renal tubular epithelial cells from camptothecin-induced apoptosis through the activation of the PI3-kinase/Akt pathway [6]. Therefore, insulin is usually used to preserve the
vitality of renal tubular epithelial cells in culture [7]. In rats with diabetes mellitus (DM), insulin treatment before IRI preserved both renal function and histomorphology, whereas insulin treatment after IRI had no such protective effects [8]. Tong et al. also proved that preconditioning with insulin improved the recovery of renal dysfunction in DM rats after kidney IRI [9]. In a normal animal model, 96 h of glucose-insulin infusion before and after IRI was found to protect the kidney against IRI. However, the glucose-insulin infusion for such a long time in rats is difficult to practice. This study did not confirm whether the glucose-insulin infusion before IRI protects the kidney against IRI. Moreover, this experiment did not explain how insulin and glucose prevent ischemic-reperfusion-induced kidney damage [10]. In the clinic, intensive insulin therapy in severely ill patients has been found to reduce the incidence of acute kidney injury by 38% and reduce the need for dialysis by 35%. Meanwhile, intensive insulin therapy increased the risk of hypoglycemia fourfold compared with conventional therapy [11, 12]. There is also a contrary finding about the renal effects of intensive insulin therapy or intensive glucose control. A meta-analysis discovered that intensive glucose control with a target glucose goal of < 6.1 mmol/L (110 mg/dL) had no effect to decrease mortality rate or dialysis requirement in severely ill adult patients but increased the incidence of hypoglycemia [13]. In a two-center, randomized controlled trial, tight glycemic control was found to reduce nosocomial infection in the postoperative period following pediatric cardiac surgery [14]. The secondary analysis of this study revealed that tight glycemic control had no effect on the reduction of cardiac surgery-associated acute kidney injury rate [15, 16]. Although the renal protection of insulin is not yet conclusive, it is still worthwhile to investigate the use of insulin to prevent IRI in the kidney, given that insulin treatment is acceptable and clinically feasible. Insulin treatment after operation may cause serious hypoglycemia. Insulin treatment before IRI may be more convenient and has little effect on the recovery from IRI. The combination of insulin and glucose may decrease hypoglycemia incidents. Therefore, we hypothesize that pretreatment with insulin and glucose could protect kidney function from IRI. The present study determined whether pretreatment with insulin and glucose could prevent IRI in mice kidneys. Moreover, the possible mechanism of insulin in kidney protection was also investigated in this study.

Methods

1. Animals

The present study was approved by the ethics committee of Zhengzhou Central Hospital of Zhengzhou University. The Guidelines of Laboratory Animal Care were strictly followed in animal experiments. Wild-type (WT) C57BL/6 male mice, 24–26 g, were commercially obtained from the animal center of Zhengzhou University. The 10% glucose drinking water was supplied to the mice during the whole study. Blood glucose concentration from the tail tip was monitored using a blood glucose meter (Sinocare Inc., Changsha, China). The first dose of insulin (Insulin glargine injection, Solostar, Beijing, China) was subcutaneously injected in the thigh region of the hindlimbs of the mice at 9:00 a.m. and the second dose of insulin was injected 12 h later. The vehicle-treated mice were subcutaneously injected with the same volume of normal saline as the insulin injection. IRI was performed on mice kidneys at 9:00 a.m., as
previously reported [17]. Briefly, anesthesia was performed by intraperitoneal injections of esketamine (50 mg/kg) and dexmedetomidine (0.25 mg/kg) [18]. The kidneys were exposed and subjected to ischemia by clamping renal pedicles with non-traumatic micro-aneurism clamps. The clamps were removed after 30 min of ischemia. The mice were kept warm throughout the procedure on a heating pad. Sham-control mice had the same surgical procedure except for pedicle clamping. The mice were sacrificed 24 h after reperfusion, and their kidneys were then harvested for Western Blot analysis and immunohistochemistry (IHC). For the pharmacological study, AKT inhibitor 40 mg/kg (3-[1-[4-(7-phenyl-3H-imidazo [4, 5-g] quinoxalin-6-yl) phenyl] methyl] piperidin-4-yl]-1H-benzimidazol-2-one, Beyotime, China) was administrated intraperitoneally 4 h before IRI in WT mice.

2. Kidney Function

Serum creatinine was measured using a commercially available creatinine assay kit (Cat No.C011-2-1, Nanjing Jiancheng Bioengineering Institute). Blood urea nitrogen (BUN) was measured as previously described (Cat No. BC1535, Solarbio, China) [19].

3. Immunohistochemistry

Immunohistochemistry was performed on paraffin-embedded kidney sections [15]. Antigen retrieving solution (0.01M sodium citrate, Ph. 6.0, Solarbio, life sciences, China) was used to retrieve the antigen from fixation. Three percent H$_2$O$_2$ was used to block endogenous peroxidase activity before blocking. After incubation with 5% normal serum, the slides were incubated with primary antibodies in a humidified chamber overnight. After washing, kidney sections were incubated with secondary antibodies, followed by ABC solution as previously reported [16]. Diaminobenzidine solution was applied to visualize the secondary antibody conjugated with horseradish peroxidase (HRP). The red substrate was used to visualize the secondary antibody conjugated with alkaline phosphatase (AP). Hematoxylin was used for nuclear staining. The sections were dehydrated with ethanol, cleared with histoclear, and then mounted using a mounting medium. NIH Image/J software (National Institutes of Health, Bethesda, MD, USA) was used to quantify the protein expression levels in the kidney.

4. Apoptosis Detection

ApopTag plus Peroxidase in situ Apoptosis Detection Kit (Cat no. MK1025, Booster Biological Technology, Ltd. China) was used to detect apoptosis. The apoptotic cells were determined using a terminal transferase dUTP nick-end labeling (TUNEL) assay. The numbers of TUNEL-positive cells per high-power field were quantified in a blinded manner.

5. Western Blot Analysis
The proteins were extracted using RIPA buffer containing a cocktail of protease inhibitors. The extracted protein concentration was determined using a Bio-Rad protein assay [21, 22]. Approximately 40 µg of protein was loaded onto SDS-polyacrylamide gels in a Tris/SDS buffer system. The proteins in gels were then transferred onto nitrocellulose membranes. After blocking, the membranes were incubated with primary antibodies overnight. After washing, the membranes were incubated with fluorescence-conjugated secondary antibodies. The proteins were detected using a western blot imaging system (Amersham™ ImageQuant™ 800 biomolecular imager, MA, USA). NIH Image/J software (National Institutes of Health, Bethesda, MD, USA) was used to quantify the protein expression.

Statistical analysis

All data are expressed as mean ± SD except tubular damage scores, which were presented as median with interquartile range. ANOVA was used to compare multiple groups followed by the Bonferroni procedure to compare means. Tubular damage scores were analyzed using the Wilcoxon rank sum test. P < 0.05 was considered statistically significant.

Results

1. The changes in the blood glucose concentration over time after treatment of insulin and glucose

To maximize insulin dose, all mice in this study were supplied with 10% glucose drinking water. Insulin was injected subcutaneously twice at an interval time of 12 h. Both 0.1 U and 0.15 U per mouse led to hypoglycemia defined as a blood glucose concentration below 4 mmol/L (Fig. 1). The doses of 0.025 and 0.05 units per mouse did not cause hypoglycemia (Fig. 1). To induce insulin action in the kidneys, an insulin dose of 0.05 units per mouse was chosen to determine whether insulin pretreatment could protect the kidney from IRI.

2. Pretreatments Of Insulin And Glucose For 24 H Protected The Kidney From IRI

The mice subjected to kidney IRI demonstrated renal dysfunction, which manifested by significant increases in BUN and serum creatinine concentration (Figs. 2A and B). Insulin pretreatment preserved the renal function from IRI. The pathological examination revealed that the renal damage in IRI was characterized by tubular dilation, sloughing of tubular epithelial cells, cast formation, and loss of the brush border in the tubular epithelial cells (Fig. 2C). The tubular damage score was used to evaluate the renal damage. Insulin pretreatment protected the kidney from pathological IRI damage through the decrease in the tubular damage score (Fig. 2D). The results suggested that insulin pretreatment protected the kidney from IRI through the preservation of renal function and morphology.

3. The pretreatment-induced protection was associated with the activation of AKT, reduction of BAX, and caspase-3 expression
Immunofluorescence analysis revealed that insulin activation AKT by phosphate AKT (P-AKT) around the nuclei of tubular epithelial cells (Fig. 3A). P-AKT would transfer from the cytoplasm to the nucleus after renal IRI. Bax—a component molecule in apoptotic signaling—was highly expressed in the nucleus of tubular epithelial cells after IRI in the kidney (Fig. 3B). Caspase-3, a critical executioner of apoptosis, exhibited in the nucleus of tubular epithelial cells in the kidney after IRI (Fig. 3C). Statistical analysis indicated that insulin-induced AKT activation could attenuate the expression of Bax and caspase-3 in the kidney after IRI (Figs. 3D-F).

Western blot analysis revealed that insulin could activate AKT by P-AKT in the kidney (Fig. 4A). The activation of AKT decreased the expression of Bax and caspase-3 after IRI in the kidney (Figs. 4A, C, and D). The western blot results were consistent with the immunofluorescence results. The results suggested that insulin pretreatment may protect the kidney from IRI through the inhibition of the apoptotic signaling pathway.

4. Pretreatments with insulin and glucose decreased IRI-induced tubular apoptosis.

To further confirm the anti-apoptosis effect of insulin, a Tunnel assay was used to detect the apoptosis in the IRI kidney (Fig. 5A). Insulin pretreatment partially reversed the renal apoptosis that was caused by IRI (Fig. 5B).

5. The Protection Of Insulin And Glucose Was Partially Reversed By An AKT Inhibitor

Based on the aforementioned results, insulin pretreatment would protect the kidney from IRI through the activation of AKT, which subsequently attenuates renal apoptosis. To further confirm the critical role of AKT in insulin-induced renal protection, an AKT inhibitor was used to assess the effect of insulin on the IRI kidney. An AKT inhibitor exacerbated renal function and damaged the IRI kidney in the presence of insulin (Figs. 6A-D). The number of apoptotic cells still worsened when an AKT inhibitor was used with insulin (Figs. 7A-B). Western blot analysis revealed that an AKT inhibitor blocked insulin-induced AKT activation (Figs. 8A-D). In the presence of insulin, an AKT inhibitor also significantly increased Bax/caspase expression and exacerbated renal apoptosis in IRI kidneys. The results suggested that insulin-induced renal protection depends on AKT activation and subsequent attenuation of apoptosis.

6. Extension of pretreatment duration failed to improve continuously the protective effect in the IRI kidney

Since insulin pretreatment protects the kidney from IRI, an extension of the pretreatment time may time-dependently improve renal protection. To confirm the effect of pretreatment time, we compared the renal protective effects of insulin pretreatment for 1, 3, and 6 days. However, there were no differences in Cre concentration, BUN concentration, and tubular damage score between 1-, 3-, and 6-day insulin pretreatments (Figs. 9A-D). There was no difference in P-AKT/Bax/caspase-3 expression between 1-, 3-,
and 6-day insulin pretreatments (Figs. 10A-D). The results indicated that a short-term insulin pretreatment (24 h) is sufficient to induce renal protection from IRI.

**Discussion**

This study presented that a 24-h pretreatment with insulin and glucose efficiently prevents IRI in the kidneys of mice. First, a proper dose of insulin per mouse for pretreatment was chosen to avoid hypoglycemia (below 4 mmol/L) through the detection of blood glucose. Pretreatment of insulin and glucose was then proved to protect the kidney through the reduction of tubular apoptosis via the P-AKT/Bax/caspase-3 signal pathway. The signaling of insulin-induced renal protection was further confirmed using an AKT inhibitor. Finally, the effects of pretreatment duration were investigated between 1-, 3-, and 6-day treatments. The 24-h pretreatment was found to have similar renal protection as either 3- or 6-day treatment (Fig. 11).

Although preconditional insulin was found renal protection in DM mice, no more research was continued in normal mice [20]. Intensive insulin therapy displayed controversial effects in renal protection in clinics but caused a definitive complication, hypoglycemia [15, 21, 22]. Fortunately, this study provided an alternative method for utilizing the renal-protective benefits of insulin by administering insulin and glucose together before renal IRI. This study systematically presented a practicable method to protect the kidney against IRI by choosing insulin doses, determining the mechanism, and optimizing treatment duration.

To enhance the effect of insulin on the kidney, we administered 10% glucose drinking water to the mice to maximize the insulin dose and avoid hypoglycemia. In this study, hypoglycemia in mice was defined as below 4 mmol/L (72 mg/dL) to minimize any adverse effect of insulin [23]. Depressive-like behaviors or neurogenic adverse effects occurred at blood glucose concentrations below 50–60 mg/dL (2.8-3.3mmol/L) [24]. However, epinephrine secretion usually occurred at blood glucose concentrations below 70 mg/dL (3.9mmol/L). Therefore, the definition of hypoglycemia was chosen as the blood glucose concentration below 4 mmol/L to adjust the insulin dose in this study.

In this study, we discovered that pretreatment with insulin and glucose protected the kidney from subsequent IRI. To elucidate the mechanism of the renal protective effect of insulin, we investigated the key component, P-AKT, in insulin signaling in this study [25]. Insulin was previously found to decrease caspase-3 activity in human renal tubular epithelial cells through PI3K/AKT signaling [6]. In an animal experiment, insulin was reported to protect the kidney after IRI through the reduction of tubular apoptosis [8]. Therefore, the signaling pathway of P-AKT/Bax/caspase-3 involving insulin and apoptosis was chosen to evaluate the renal protection of insulin treatment before IRI. Immunofluorescence results indicated that insulin increased P-AKT expression around the nucleus, which subsequently enters into the nucleus in tubular cells after IRI. The insulin-activated P-AKT may inhibit the apoptosis of tubular epithelial cells through a decrease in the expression of Bax and cleaved caspase-3 in the nucleus. The western blot result was consistent with the immunofluorescence results in the expression of P-
AKT/Bax/caspase-3 signaling. The role of P-AKT/Bax/caspase-3 signaling in insulin-induced renal protection was confirmed using an AKT inhibitor in this study.

Pretreatment with insulin and glucose before IRI is easy to practice in clinical conditions. To fine-tune the pretreatment with insulin and glucose, we investigated whether extending the pretreatment time can continuously boost insulin-induced renal protection. Surprisingly, there were no differences in renal protection between 0-, 1-, 3-, and 6-day pretreatments. It was interpreted that a short-term 24-h pretreatment is enough to induce renal protection. This finding facilitates the pretreatment with insulin and glucose in clinical conditions where IRI in the kidney usually occurs, such as heart and large vessel surgeries. A further clinical trial is warranted to confirm the renal protection induced by insulin and glucose, given that the pretreatment is acceptable and practicable without any damage.

Conclusion

A short-term pretreatment with insulin and glucose protected the kidney from IRI through activation of p-AKT and subsequent reduction of the BAX-caspase-3-induced apoptosis. The short-term pretreatment provides a practicable method for protecting the kidney from predicable IRI.

Declarations

Ethics approval and consent to participate

This study was approved by the ethics committee of Zhengzhou Central Hospital of Zhengzhou University.

Consent for publication

All authors agreed to the final terms.

Competing interests

The authors declare no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Authors' contributions

Credit Author Statement: QC, XJ: Conceptualization; LS, CZ, HB, LL, HL: Methodology; LS, CZ, HB, LL, HL: Software; QC, XJ, HL: Validation; XJ: Formal analysis; LS, CZ, HB, LL, HL: Investigation; XJ: Resources; XJ,
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Availability of data and materials

The data and material are available from the corresponding author if a proper quest is received.

References


Figures
The blood glucose concentration changed over time after the subcutaneous injection of insulin in the mice supplied with 10% glucose drinking water. Hypoglycemia was defined as a blood glucose concentration below 4 mmol/L (72 mg/dL) in this study.

Figure 1
Figure 2

Combination pretreatment of 0.05-unit insulin injection and 10% glucose drinking water protected the kidney from IRI. A. Summarized data showing the changes in serum Cre concentration among the four groups (Sham surgery with vehicle injection, IRI surgery with vehicle injection, Sham surgery with insulin injection, and IRI surgery with insulin injection). **P < 0.01, compared with the vehicle-treated sham group. &&P < 0.01, compared with the vehicle-treated IRI group. B. Summarized data showing the changes in serum BUN concentration among the four groups. **P < 0.01, compared with the vehicle-treated sham group. &&P < 0.01, compared with the vehicle-treated IRI group. ##P < 0.01, compared with the vehicle-treated IRI group. C. Representative renal H&E staining images for the four groups. D. Summarized tubular damage score of the four groups. **P < 0.01, compared with the vehicle-treated sham group. &&P < 0.01, compared with the vehicle-treated IRI group. ##P < 0.01, compared with the vehicle-treated IRI group.
Figure 3

Immunofluorescence results of the expression of P-AKT, Bax, and caspase-3 in the kidneys. A. P-AKT expression in the kidneys of the four groups (Sham surgery with vehicle injection, IRI surgery with vehicle injection, Sham surgery with insulin injection, and IRI surgery with insulin injection). P-AKR was stained red with Cy5.5. E-Cadherin was stained green with FITC in the cytoplasm of tubular epithelial cells. The nucleus was stained purple with DAPI. B. BAX expression in the kidneys of the four groups. BAX was stained red with Cy5.5. E-Cadherin was stained green with FITC. The nucleus was stained purple with DAPI. C. Caspase-3 expression in the kidneys of the four groups. Caspase-3 was stained red with Cy5.5. E-Cadherin was stained green with FITC. The nucleus was stained purple with DAPI. D. Quantitative analysis of P-AKT expression in the kidneys of the four groups. **P < 0.01, compared with the vehicle-treated sham group. #P < 0.01, compared with the vehicle-treated IRI group. E. Quantitative analysis of BAX expression in the kidneys of the four groups. **P < 0.01, compared with the vehicle-treated sham group. &P < 0.05, compared with the vehicle-treated IRI group. ##P < 0.01, compared with the vehicle-treated IRI group. F. The quantitative analysis of caspase-3 expression in the kidney of the four groups.
**P < 0.01, compared with the vehicle-treated sham group. &&P < 0.01, compared with the vehicle-treated IRI group.

**Figure 4**

Western blot results of P-AKT, Bax, and caspase-3 in the kidneys. A. Representative results of P-AKT, Bax, and caspase-3 expression in western blot results of the four groups (Sham surgery with vehicle injection, IRI surgery with Vehicle injection, Sham surgery with Insulin injection, and IRI surgery with insulin injection). B. Quantitative analysis of P-AKT expression in the western blot. **P < 0.01, compared with the vehicle-treated sham group. &&P < 0.01, compared with the vehicle-treated IRI group. C. Quantitative analysis of Bax expression in the western blot. **P < 0.01, compared with the vehicle-treated sham group. &&P < 0.01, compared with the vehicle-treated IRI group. ##P < 0.01, compared with the insulin-treated IRI group. D. Quantitative analysis of cleaved caspase-3 expression in the western blot. **P < 0.01, compared with the vehicle-treated sham group. ##P < 0.01, compared with the vehicle-treated IRI group. &P < 0.05, compared with the vehicle-treated IRI group.
Figure 5

Apoptosis in the kidney after IRI using TUNEL assay. A. Representative results of apoptosis in the kidneys with apoptotic cells (brown) counterstaining with hematoxylin (blue) of the four groups (Sham surgery with vehicle injection, IRI surgery with vehicle injection, Sham surgery with Insulin injection, and IRI surgery with insulin injection) (original magnification is 400×). (B) The apoptotic cells were quantitatively analyzed in the kidneys. **P < 0.01 compared with the vehicle-treated sham group; ##P < 0.01 compared with the vehicle-treated IRI group. &&P < 0.01, compared with the vehicle-treated IRI group. n = 6 per group. HPF means high power field.
Figure 6

P-AKT inhibitor reversed the protection of insulin and glucose in the IRI kidney. A. Summarized data showing the changes in serum Cre concentration of the four groups (Sham surgery with insulin injection, IRI surgery with insulin injection, Sham surgery with Insulin-and-AKT inhibitor injection, and IRI surgery with insulin-and-AKT inhibitor injection). *P < 0.05, compared with the insulin-treated sham group. && P < 0.01, compared with the vehicle-treated sham group. # P < 0.05, compared with the insulin-treated IRI group. B. Summarized data showing the changes in serum BUN concentration in mice of the four groups. ** P < 0.01, compared with the insulin-treated sham group. &&& P < 0.01, compared with the insulin-treated IRI group. C. Representative renal H&E staining images of the four groups. D. Summarized tubular damage score of the four groups. ** P < 0.01, compared with the vehicle-treated sham group. &&& P < 0.01, compared with the vehicle-treated IRI group. ## P < 0.01, compared with the vehicle-treated IRI group.
Figure 7

P-AKT inhibitor reversed the insulin-induced improvement in apoptosis in the IRI kidney. A. Representative results of apoptosis in the kidneys with apoptotic cells (brown) counterstaining with hematoxylin (blue) (original magnification is 400×) of the four groups (Sham surgery with insulin injection, IRI surgery with insulin injection, Sham surgery with Insulin-and-AKT inhibitor injection, and IRI surgery with insulin-and-AKT inhibitor injection). B. The apoptotic cells were quantitatively analyzed in the kidneys. **P < 0.01 compared with the mice treated with Sham surgery and insulin injection; ##P < 0.01 compared with the mice treated with IRI surgery and insulin and AKT inhibitor injection. &&P < 0.01, compared with the mice treated with IRI surgery and insulin injection. n = 6 per group. HPF means high power field.
Figure 8

Western blot results of P-AKT, Bax, and caspase-3 expression in the kidney after IRI in the presence of insulin and AKT inhibitor. A. Representative results of P-AKT, Bax, and caspase-3 expression in western blot results of the four groups (Sham surgery with insulin injection, IRI surgery with insulin injection, Sham surgery with Insulin-and-AKT inhibitor injection, and IRI surgery with Insulin-and-AKT inhibitor injection). B. Quantitative analysis of P-AKT expression in the western blot. **P < 0.01, compared with the sham group treated with insulin and AKT inhibitor. ##P < 0.01, compared with the IRI group treated with insulin and AKT inhibitor. C. Quantitative analysis of Bax expression in the western blot. **P < 0.01, compared with the insulin-treated sham group. && P < 0.01, compared with the insulin-treated IRI group. ##P < 0.01, compared with the IRI group treated with insulin and AKT inhibitor. D. Quantitative analysis of cleaved caspase-3 expression in the western blot. **P < 0.01, compared with the insulin-treated sham group. ##P < 0.01, compared with the IRI group treated with insulin and AKT inhibitor. &&P < 0.01, compared with the insulin-treated IRI group.
Figure 9

The mice were pretreated with insulin for varying periods (1 d, 3 d, and 6 d, Twice a day 0.5 U per time). The mice received the same treatment without renal pedicle clamping was used as a sham surgery. A. Summarized data showing the effects of insulin on Cre changes in mice with/without IRI. **P < 0.01, compared with the vehicle-treated sham group. &&P < 0.01, compared with the vehicle-treated IRI group. ##P < 0.01, compared with the vehicle-treated IRI group. B. Summarized data showing the effects of insulin on BUN changes in mice with/without IRI. **P < 0.01, compared with the vehicle-treated sham group. &&P < 0.01, compared with the vehicle-treated IRI group. @@P < 0.01, compared with the vehicle-treated IRI group. C. Summarized tubular damage score in C57/B6 mice with or without ischemia /reperfusion. **P < 0.01, compared with the vehicle-treated sham group. &&P < 0.01, compared with the vehicle-treated IRI group. ##P < 0.01, compared with the vehicle-treated IRI group. @@P < 0.01, compared with the vehicle-treated IRI group. C. Representative renal HE staining images for the insulin-injected mice for 0, 1, 3, and 6 days. The mice were subjected to sham or IRI surgery.
Figure 10

The P-AKT, Bax, and caspase-3 expression in western blots after different pretreatment durations of insulin and glucose. A. Representative results of P-AKT, Bax, and caspase-3 expression in Western blot in insulin-treated (insulin injection for 0, 1, 3, and 6 days) mice that were subjected to sham or IRI kidney surgery. B. Quantitative analysis of P-AKT expression in the western blot. **P < 0.01, compared with the vehicle-treated sham group. ##P < 0.01, compared with the vehicle-treated IRI group. C. Quantitative analysis of Bax expression in the western blot. **P < 0.01, compared with the vehicle-treated sham group. ##P < 0.01, compared with the vehicle-treated IRI group. &&P < 0.01, compared with the vehicle-treated IRI group. D. Quantitative analysis of cleaved caspase-3 expression in the western blot. **P < 0.01, compared with the vehicle-treated sham group. $$$P < 0.01, compared with the vehicle-treated IRI group. ##P < 0.01, compared with the vehicle-treated IRI group.
Figure 11

The flow chart of research methodology