Resuscitative Endovascular Balloon Occlusion of the Aorta in a Normovolemic Rabbit Model: a Pilot Study

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Research

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Abstract

Background: The purpose of this study was to verify physiological, end-organ and systemic inflammatory changes in zone II after resuscitative endovascular balloon occlusion of the aorta (REBOA) in a normovolemic rabbit model.

Methods: Anaesthetized rabbits were subjected to aortic balloon occlusion for different times (15 min, 30 min, 60 min and 90 min) followed by 2 h of reperfusion. Rabbits with no balloon occlusion were set as the control group. ELISAs were used to examine the serum levels of ALT, AST, Cr, BUN, MDA, SOD, IL-8, IL-6, and TNF-α; HE staining was used to identify the morphological changes in the kidney; RT-PCR was used to detect the mRNA levels of IL-6, IL-8, TNF-α and NF-κB in the kidney and uterus; and Western blotting was used to measure the protein expression levels of IL-6, IL-8, TNF-α and NF-κB in the kidney and uterus.

Results: Plasma concentrations of liver markers, kidney markers, inflammatory factors and oxidative stress indicators were significantly increased at the end of reperfusion in the 30 min, 60 min and 90 min groups. Damage to the kidney occurred in the 30 min, 60 min and 90 min groups. The mRNA and protein expression levels of IL-6, IL-8, TNF-α and NF-κB in the kidney and uterus were significantly increased at the end of reperfusion in the 30 min group, and as the time of occlusion extended, these levels continued to increase.

Conclusion: Activation of systemic inflammation and ischaemia-reperfusion injury of end-organs occurred when the occlusion time reached 30 min. Therefore, 15 min should be regarded as a safe period of REBOA in zone II.

Background

Resuscitative endovascular balloon occlusion of the aorta (REBOA) is an effective technology to address noncompressible torso haemorrhage. The procedure involves placing and inflating a balloon catheter in the abdominal aorta under the guidance of imaging equipment. This technique can effectively prevent and reduce fatal bleeding during surgery, ensuring that abdominal surgery, urological surgery, vascular surgery, organ transplantation, tumour resection, obstetrics and gynaecology procedures, and pelvic and orthopaedic surgeries can start in a clear field [1].

The aortic occlusion area is often divided into three zones: zone I, comprising the left subclavian artery opening to the celiac artery (upper abdominal aorta); zone II, comprising the celiac artery to the lowest renal artery (middle abdominal aorta); and zone III, comprising the lowest renal artery to the bifurcation of the abdominal aorta (lower abdominal aorta). At present, there are many debates regarding the clinical application of REBOA. Our former clinical studies confirmed that REBOA at the level of the renal artery resulted in less blood loss [2]. The main reasons for balloon occlusion in this area lie in the presence of natural collateral circulations to the uterus from the ovarian arterial blood supply [3]. Kim et al. reported that the frequency of postpartum haemorrhage originating from the ovarian arteries reached 11.8% [4].

In clinical practice, there is no uniform standard for the duration of blood flow occlusion. A longer period of REBOA will cause ischaemia-reperfusion injury (IRI), which will lead to organ failure. The pathophysiology of IRI is related to microvascular dysfunction, which is characterized by telangiectasia, decreased tissue perfusion, and organ failure [5]. With the extension of REBOA time, the burden of IRI increases, and organ failure occurs [6]. The organs and tissues below the level of occlusion will produce a large number of inflammatory mediators, such as endotoxin, oxygen free radicals, and complement activation of tumour necrosis factor α (TNF-α) and interleukin-1β (IL-1β) due to ischaemia and reperfusion, and other cytokines. These mediators can release large amounts of oxygen free radicals and proteolytic enzymes after entering multiple tissues in the body from the bloodstream, causing tissue damage. In addition, activating cytokines and inflammatory mediators such as interleukin-6 (IL-6), interleukin-8 (IL-8), TNF-α and IL-1β further stimulate and exacerbate the inflammatory cascade. Thus, the REBOA time should strike a reasonable balance between the physiological haemodynamic effects of obstructing blood flow to organs and the time required to effectively control bleeding.

To date, various clinical studies and haemorrhagic animal models have been performed to describe the effects of REBOA in a state of haemorrhagic shock [7–9], but few studies have been conducted to verify the effects of REBOA in normovolemic conditions and provide a thorough description of the changes from 15 min to 90 min of balloon occlusion.

Therefore, the purpose of this study was to investigate changes in physiology, IRI and systemic inflammation in response to different times of REBOA in zone II in a non-shock rabbit model.

Materials And Methods

Animals

The animal instrumentation and ensuing experiments were approved by the institutional review board for animal research. Our study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Zhengzhou University First Affiliated Hospital. Forty female New Zealand white rabbits (Hualan Biological Co., Ltd. China) (2.5–3.5 kg) were housed under conditions with a 12 h dark/light cycle and free access to food and water before experimentation.

Animal preparation

Before intervention, the animals were randomized into five groups: I (control group, no occlusion), II (occlusion for 15 min), III (occlusion for 30 min), IV (occlusion for 60 min), and V (occlusion for 90 min). The hair on the right inguinal region and ventral side of the chest and abdomen was shaved, and the skin was sterilized. Animals were anaesthetized by intravenous (i.v.) pentobarbital sodium 20–30 mg/kg. The core body temperature of rabbits was kept at 37°C throughout experiments with heating pads. The zone II aorta was occluded with a balloon catheter for different times and then reperfused for 2 h. Then, all animals were sacrificed with air injection via the ear marginal vein before the collection of sera, renal tissues and uterine tissues.

Experimental protocol

A 2F Fogarty balloon catheter (Guorui Jiachuang Technology Co., Ltd. China) was inserted through the femoral artery sheath, and the balloon position was adjusted with digital subtraction angiography (DSA) ensuring that the upper edge of the balloon was placed against the opening of the higher renal artery. Indirect abdominal aortic angiography was performed through the right ear vein to confirm the effect of occlusion. After the occlusion time was reached, the balloon was slowly deflated immediately to allow 2 h of blood reperfusion, at which time the rabbits were sacrificed to collect serum, renal tissue and uterine tissue.

Evaluation of hepatic and renal function and inflammatory factors

Whole blood from rabbits was centrifuged for 10 min at 3000 rpm/min, and then the serum layer was collected for the detection of the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum creatinine (Scr) and blood urea nitrogen (BUN), which were examined by the laboratory in our hospital. The levels of MDA, SOD, IL-8, IL-6, and TNF-α in the serum were detected with ELISA kits.

Histopathology

The renal samples were fixed in 4% paraformaldehyde for 12 h followed by paraffin embedding, sectioning and staining. Histomorphological evaluation was performed by the pathologist using a double-blind method.

Determination of the mRNA levels of IL-6, IL-8, TNF-α and NF-κB in the kidney and uterus

Total RNA was extracted using TRIzol (Aidlab) following the manufacturer's instructions. cDNA was synthesized with the HiScript Reverse Transcription kit (VAZYME). The primers used are shown in Table 1. Reverse transcription for RT-PCR was carried out with the following program: 95°C for 10 min followed by 40 cycles of 95°C for 30 s, 50°C for 2 min and 60°C for 30 s. The data were calculated by the 2-ΔΔCt method as described in the instructions.

Western blot of IL-6, IL-8, TNF-α and NF-κB in the kidney and uterus

The tissues were homogenized, and the supernatants were collected to examine the protein concentrations by the BCA method. Proteins were separated by SDS-PAGE and transferred to polyvinylidene fluoride membranes. The membranes were then incubated with antibodies against IL-6 (ABBEXA, UK, 1:500), NF-κB (Lsbio, USA, 1:500), TNF-α (Santa, USA, 1:300), IL-8 (Lsbio, USA, 1:300), and β-actin (Boster, China, 1:200) overnight at 4°C, followed by incubation with an HRP-conjugated secondary antibody (Abmart, Shanghai, China, 1:3000) at 37°C for 2 h. The reactive bands were visualized using ECL-Plus reagent (Thermo, USA). The density of the bands was quantified by ImageJ software (USA).

Statistical analysis

Statistical analysis was performed with SPSS 19.0 statistical software. Measurement data are expressed as the means ± standard deviation ($\bar{x}\pm s$). Comparisons among three groups or more were performed by one-way ANOVA. The test level was α = 0.05, and $P<0.05$ was considered statistically significant.

Results

1 Animal models

All experimental rabbits were successfully modelled. Delayed angiography under DSA showed that blood flow in the lower abdominal aorta below the level of balloon occlusion was completely interrupted, as there was no contrast agent visible below the balloon catheter (Figure 1).

2 Liver and kidney function indicators

After the aortas were occluded for the indicated times in each group, the BUN levels in groups IV and V were 7.93 ± 1.12 mmol/L and 9.67 ± 1.63 mmol/L, respectively, which were significantly higher than those in group I (4.93±0.39 mmol/L, $P<0.05$ for both). The Scr levels in groups IV and V were significantly higher than those in group I (58.86 ± 8.21 and 68.71 ± 5.82 mmol/L vs 50.0±3.91 mmol/L; $P<0.05$ for both). AST levels in groups IV and V increased significantly to 60.8±9.44 U/L and 76.85 ± 6.59 U/L, respectively, compared with that in group I (21.28 ± 4.03 U/L; $P<0.01$ for both). The ALT levels in groups III, IV and V were 29.0 ± 1.82 U/L, 52.14 ± 4.37 U/L, and 80.28 ± 4.99 U/L, respectively, which were significantly higher than that in group I (24.71±4.27 U/L; $P<0.05$ for all; Figure 2).

3 Inflammatory factors

After 2 h of reperfusion, the IL-6, IL-8, and TNF-α levels in groups III, IV and V were all increased compared with those in group I. The concentrations of IL-6 in groups III, IV and V were 24.6±1.73 pg/mL, 34.41±3.33 pg/mL and 42.58±3.14 pg/mL, compared with 15.41±1.0 pg/mL for group I ($P<0.01$ for all); the IL-8 concentrations in groups III, IV, and V were 172.15±20.65 pg/mL, 209.15±27.3 pg/mL, 289.03 ± 18.69 pg/mL, respectively, compared with 85.62±9.66 pg/mL for group I ($P<0.01$ for all); and the TNF-α concentrations in groups III, IV and V were 182.28±17.72 pg/mL, 232.43±19.32 pg/mL and 323.86±13.47 pg/mL, respectively, compared with 127.14±10.23 pg/mL for group I ($P<0.01$ for all; Figure 3).

4 Oxidative stress indicators among groups

The MDA levels in groups III, IV and V increased to 3.17±0.28 nmol/mL, 3.59±0.23 nmol/mL and 4.91±0.28 nmol/mL, respectively, compared with the level in group I (1.61±0.12 nmol/mL; $P<0.01$ for all); however, the SOD levels in groups III, IV and V gradually decreased to 138.0±13.21U/mL, 112.71±7.47U/mL and 87.0±9.63U/mL, in comparison with the level in group I (163±6.74 U/mL), and the difference was statistically significant ($P<0.01$ for all; Figure 4).

5 The pathology of kidney tissue

The kidney tissue structure in groups I and II was clear, with the structure of the glomerular cells and renal tubular cells showing mainly normal features. In group III, mild swelling of glomerular cells and diffuse swelling of renal tubular cells were observed. In group IV, severe diffuse swelling and some necrosis of the renal tubular cells was observed, and moderate to severe swelling of glomerular cells was seen. In group V, the degree of swelling of the glomerular cells and renal tubular cells was more severe, and haemorrhage was present in the renal interstitium (Figure 5).

6 The expression levels of IL-6, IL-8, TNF-α and NF-κB mRNA in kidney and uterine tissues

6.1 Kidney tissues: IL-6 mRNA levels in groups III, IV and V were 1.267±0.052, 2.221±0.258 and 2.749±0.142, respectively, compared with those in group I (0.884±0.026), the differences were statistically significant ($P<0.01$ for all). IL-8 mRNA levels in groups III, IV and V were higher than those in group 1 (1.402±0.073, 2.056±0.241 and 2.269±0.374, respectively, vs. 0.857±0.126), and the differences were statistically significant ($P<0.05$, all). TNF-α mRNA levels in groups III, IV and V were 1.486±0.105, 1.987±0.211 and 2.954±0.176, respectively, which were higher than those in group I (0.823±0.089), and the differences were statistically significant ($P<0.05$, all). Finally, the NF-κB mRNA levels in groups III, IV and V were 1.636±0.279, 2.330±0.643 and 3.243±0.274, which were higher than those in group I (0.958±0.069), the differences were statistically significant ($P<0.05$ for all). The data are summarized in Figure 6.

6.2 Uterine tissues: The IL-6 mRNA levels in groups III, IV and V were 1.278±0.088, 1.635±0.174 and 2.028±0.091, respectively, which were higher than those in group I (0.947±0.084), and the differences were statistically significant ($P<0.05$ for all). IL-8 mRNA levels in groups III, IV and V were 1.357±0.159, 1.852±0.121 and 2.232±0.103, respectively, which were higher than those in group I (0.979±0.041), and the differences were statistically significant ($P<0.05$, all). TNF-α mRNA levels in groups III, IV and V were 1.470±0.208, 1.626±0.242 and 2.113±0.069, respectively, which were higher than those in group I (0.987±0.062), and the differences were statistically significant ($P<0.05$, all). Finally, NF-κB mRNA levels in groups III, IV and V were 1.770±0.118, 2.105±0.097 and 2.542±0.098, respectively, which were higher than those in group I (0.944±0.073), and the differences were statistically significant ($P<0.05$ for all). The data are summarized in Figure 7.

7 The protein expression levels of IL-6, IL-8, TNF-α and NF-κB in the kidney and uterine tissues

7.1 Kidney tissues: IL-6 protein expression in groups III, IV and V was 0.446±0.064, 0.592±0.046 and 0.673±0.062, respectively, which was higher than that in group I (0.317±0.024), and the differences were statistically significant ($P<0.05$ for all). IL-8 protein expression in groups III, IV and V was 0.427±0.007, 0.581±0.021 and 0.640±0.165, respectively, which was higher than that in group I (0.301±0.029), and the differences were statistically significant ($P<0.05$ for all). TNF-α protein expression in groups III, IV and V was 0.583±0.090, 0.738±0.070 and 0.844±0.086, respectively, which was higher than that in group I (0.342±0.067), and the differences were statistically significant ($P<0.05$ for all). Finally, NF-κB protein expression in groups III, IV and V was 0.556±0.051, 0.713±0.068 and 0.802±0.079, respectively, which

was higher than that in group I (0.314±0.039), and the differences were statistically significant (P<0.05 for all). The data are summarized in Figure 8.

7.2 Uterine tissues: IL-6 protein expression in groups III, IV and V was 0.284±0.425, 0.426±0.055 and 0.487±0.091, respectively, which was higher than that in group I 0.154±0.034, and the differences were statistically significant (P<0.05 for all); IL-8 protein expression in groups III, IV and V was 0.179±0.033, 0.286±0.013 and 0.349±0.032, respectively, which was higher than that in group I (0.118±0.016), and the differences were statistically significant (P<0.05 for all). TNF-α protein expression in groups III, IV and V was 0.537±0.025, 0.661±0.038 and 0.773±0.024, respectively, which was higher than that in group I (0.329±0.001), and the differences were statistically significant (P<0.05 for all). Finally, NF-κB protein expression in groups III, IV and V were 0.666±0.033, 0.892±0.041 and 0.979±0.039, respectively, which was higher than that in group I (0.454±0.009), and the differences were statistically significant (P<0.05 for all). The data are summarized in Figure 9.

Discussion

Until now, there has been no general consensus regarding the optimal occlusion time of the upper and middle abdominal aorta. Ciscato JG et al used vascular clips to clamp the abdominal aorta above the trunk of the celiac artery in 27 dogs and found that 60 min of occlusion followed by 30 min of reperfusion did not cause IRI of kidney tissue [10]. Other scholars, such as Chao CS, found that when the occlusion time of the zone II abdominal aorta in pigs exceeded 30 minutes with a balloon catheter, the intra-abdominal pressure increased, pathological changes in multiple organs occurred, and the mixed venous oxygen saturation was reduced [11]. Athanasiadis D et al concluded that Scr levels increased 30 min after abdominal aorta occlusion above the celiac trunk in pigs with open aneurysms [12].

The results of this study showed that the BUN and Scr of rabbits were increased after ischaemia for 60 min followed by reperfusion for 2 h, and ALT increased significantly after ischaemia for 30 min followed by reperfusion for 2 h. The pathological results of the kidney in this experiment confirmed that swelling of glomerular cells and diffuse swelling of renal tubular cells appeared when the occlusion time reached more than 30 min. The degree of damage increased as the occlusion time increased. However, no IRI of pathological tissues appeared after 30 min of occlusion followed by reperfusion for 2 h. Therefore, it could be concluded that 30 min of occlusion was not safe because IRI occurred. This is basically consistent with the view of Yasuhito et al, who blocked the renal artery to perform a kidney-sparing renal tumour resection, and diffuse and irreversible damage of the kidney occurred when the ischaemia time reached 25 min [13].

IRI occurs when tissues or organs are subjected to ischemia for a certain period of time followed by restoration of blood flow or oxygen supply. The pathophysiological changes mainly included the production of oxygen free radicals, inflammatory factors, and lipid peroxidation metabolites. SOD, an important antioxidant enzyme in the body, is a metalloproteinase that can protect cells from damage due to oxygen free radicals. As a product of lipid peroxidation, MDA reflects the level of oxidative stress in the

body. The results of our experiment showed that when the occlusion time was longer than 30 min, injuries occurred after reperfusion for 2 h, which were reflected by a significant increase in MDA levels and a severe decrease in SOD activity. Aslan M et al. also reported that when an IR model was established in mice, MDA levels increased and SOD activity decreased significantly in the uterus and ovaries [14].

When ischaemia-reperfusion was achieved, tissues were more vulnerable to inflammation. Cytokines, chemokines and neutrophils play an important role in this process. A large number of inflammatory mediators and cytokines, such as IL-1, IL-6 and TNF-α, are released after activation of neutrophils and endothelial cells [15]. In this experiment, the mRNA expression and protein levels of IL-6, IL-8, TNF-α and NF-κB increased as the occlusion time increased extended. After more than 30 min of occlusion, significant expression differences in the kidneys, uterus and ovaries were observed in those values after reperfusion for 2 h. This study is consistent with the results reported by Nayki C and others that IL-1β and TNF-α levels increased significantly after ovarian ischaemia-reperfusion [16]. Many studies have shown that when ischaemia-reperfusion occurs, the levels of IL-6, IL-8 and TNF-α are all significantly increased [17, 18]. NF-κB can induce the gene expression of a variety of cytokines, adhesion molecules and chemokines; regulate inflammation upstream; and promote the occurrence of I/R injury [15, 19]. NF-κB can regulate the production of cytokines, including TNF-α, IL-1β and IL-6, at the transcriptional level [20]. The results of this experiment showed that when the abdominal aorta was blocked for more than 30 min followed by 2 h of reperfusion, the mRNA and protein levels of NF-κB in the distal tissues increased significantly. Liu G et al confirmed in mice that when myocardial ischaemia-reperfusion occurs, the expression level of NF-κB increases and regulates the expression of a variety of inflammatory factors, including increases in IL-1β and TNF-α expression [21].

There are some limitations to our experimental model. First, the short reperfusion time is not long enough to allow investigation of long-term damage to organs. Second, the number of animals used was small, and further study with a large cohort of rabbits may be needed.

A longer period of occlusion may cause irreversible damage to the body, and the risk of organ necrosis simultaneously increases. When the occlusion is removed, the metabolites accumulated in the ischaemic tissues immediately enter the systemic circulation, which will further aggravate the damage to the organs. Therefore, it is necessary to strictly control the occlusion time to avoid serious damage to the body's multiple organ functions.

Conclusions

As long as the occlusion time was limited to 15 minutes, no significant IRI the distal tissues and organs occurred. Therefore, 15 minutes should be regarded as the safe occlusion time.

List Of Abbreviations

REBOA, Endovascular Balloon Occlusion in Aorta; SOD, Superoxide dismutase; MDA, Malondialdehyde; IL-6, Interleukin 6; IL-8, Interleukin 8; TNF-α, Tumor Necrosis Factor α; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; Scr, Serum creatinine; BUN, Blood urea nitrogen; NF-κB, Nuclear Factor kappa-B; HIF-1α, Hypoxia Inducible Factor-1α; I/R, Ischemia-Reperfusion.

Declarations

Ethics approval and consent to participate

The animal instrumentation and ensuing experiments were approved by the institutional review board for animal research. Our study protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Zhengzhou University First Affiliated Hospital

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request

Competing interests

The authors have no conflicts of interest to declare.

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There is no funding related to this study.

Authors' contributions

Juanfang Liu was a major contributor in writing the manuscript and a designer in experiment. and interpreted the animal data. Jianhao Zhang performed the interventional procedure of rabbits. Shanshan Xie collected and interpreted the animal data. Yingxia Liu performed the histological examination of the kidney. Xueliang Zhou did the bio-experiment. Zhaonan Li analyzed the animal data. Xinwei Han organized the whole experiment and rewrote the manuscript. All authors read and approved the final manuscript.

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Table

Table 1

Primers used for real-time PCR analysis

Name	Species	Sense strand sequence	Anti-sense strand sequence
β-actin	Rabbit	TGGCTCTAACAGTCCGCCTAG	AGTGCGACGTGGACATCCG
TNF-α	Rabbit	GTCAACCTCCTCTCTGCCAT	ATCCCAAAGTAGACCTGCCC
IL-6	Rabbit	TACCGCTTTCCCCACTTCAG	CAGATTGACTTCCGCCAGTG
IL-8	Rabbit	TACAGAGCTTCGATGCCAGT	CCTTCTGCACCCACTTTTCC
NF-κB	Rabbit	TCAGCAGGCACCAGTTCTAA	ACCCATACGCAAAGGAGTCA

Figures

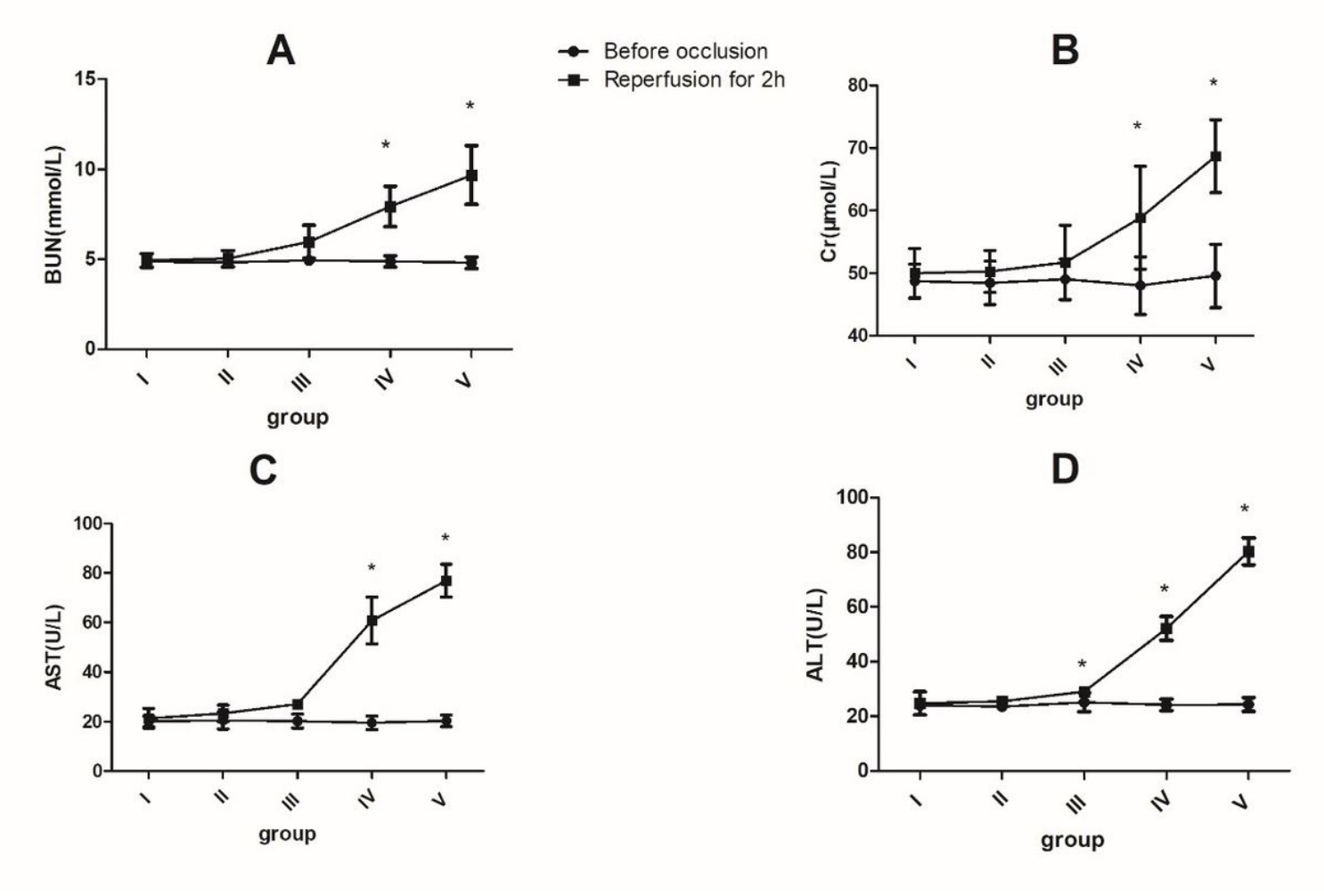

Figure 2

Hepatic and renal function among groups before occlusion and after 2 h of reperfusion * P<0.05

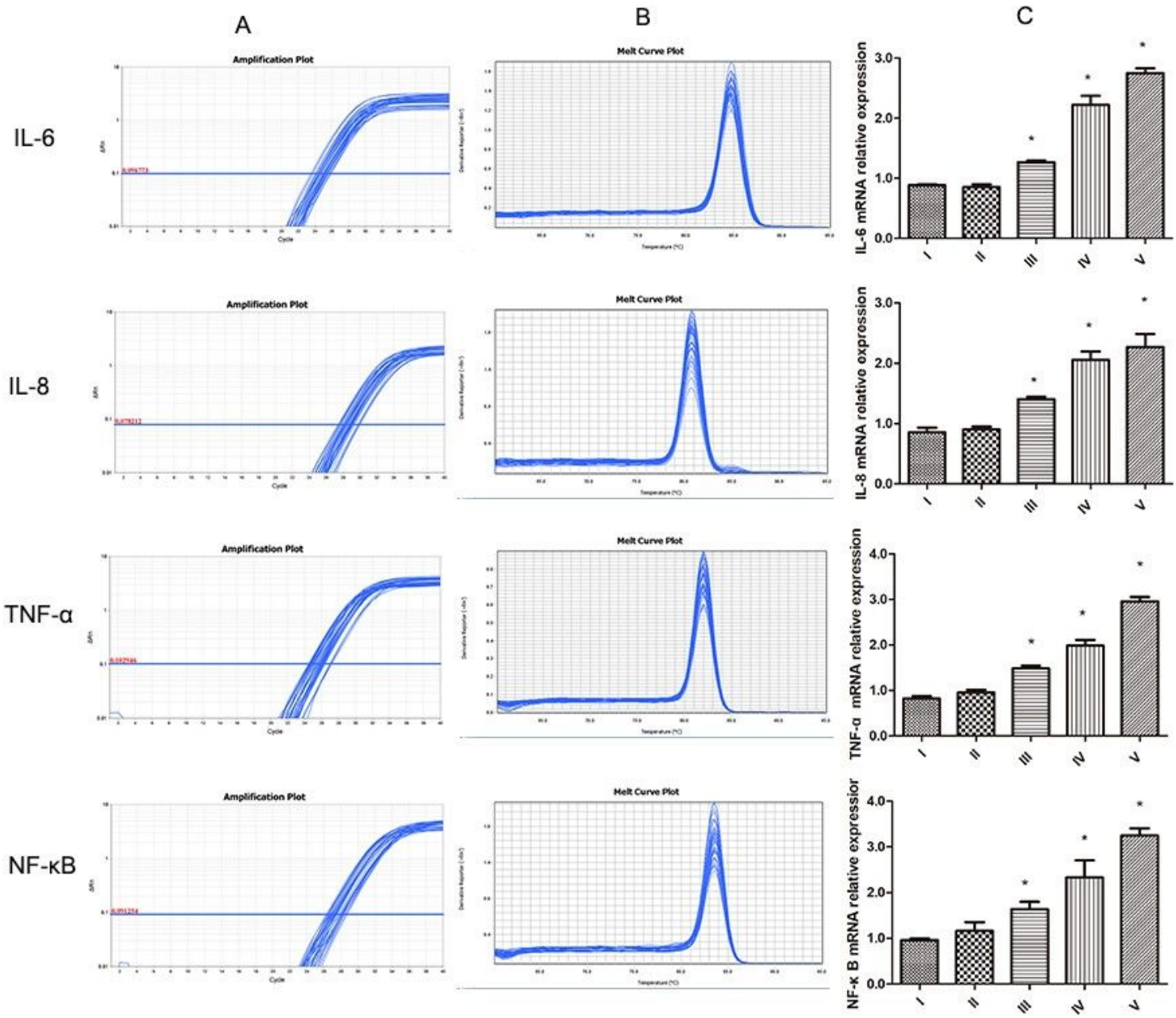

Figure 6

Fluorescence values of quantitative PCR results of renal tissue A: amplification curve, B: dissolution curve, C: relative expression level of mRNA, *indicates that the difference is statistically significant compared with group I, $P<0.05$.