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Resveratrol Reduces Kidney Injury in a Rat Model of Uremia and is Associated with Increased Expression of Heat Shock Protein 70 (Hsp70)

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Manuscript Preparation E
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Background: This study aimed to investigate the effects of resveratrol on kidney function in a rat model of uremia and the expression of heat shock proteins.

Material/Methods: The rat model of uremia was developed by 5/6 nephrectomy of Sprague–Dawley rats. The Hsp70 inhibitor MKT-077, a rhodacyanine dye, was used. The study groups included rats with sham surgery (the sham group), the rat model of uremia (the model group), the solvent-treated control group (the control group), the rat model treated with resveratrol group (the resveratrol group), the rat model treated with MKT-077 (the MKT-077 group), and the resveratrol+MKT-077 group. Kidney tissues were studied histologically. Renal cell apoptosis was detected by the TUNEL method. Expression of p53, Bax, and Bcl-2 mRNA and protein were detected by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and immunohistochemistry, respectively.

Results: Compared with the sham group, the expression levels of heat shock proteins Hsp70, Hsp90, Hsp27, Hsp25, Hsp40, and Hsp60 in the kidney of the rat model group increased to different degrees. Compared with the model group, the Hsp70 levels in the resveratrol group were significantly increased ($p<0.05$). Compared with the model group, treatment with MKT-077 reduced the survival rate of rats, which was increased following resveratrol treatment. Compared with the resveratrol group, renal function in the resveratrol+MKT-077 group was significantly reduced ($p<0.05$).

Conclusions: In a rat model of uremia, resveratrol reduced renal injury and improved both renal function and survival, which were associated with increased expression of Hsp70.

MeSH Keywords: Acute Kidney Injury • HSP70 Heat-Shock Proteins • Uremia

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Background

Uremia results from kidney injury and can also promote the progression of chronic renal failure by damaging renal tubular epithelial cells [1,2]. Chronic kidney disease is a risk factor for both renal disease and cardiovascular disease [3,4]. Chronic kidney disease reduces patient quality of life and is associated with increased patient mortality [5]. Therefore, it is important to continue to investigate potential treatments to prevent chronic kidney injury and reduce kidney inflammation [6].

Resveratrol, or 3,5,4'-trihydroxystilbene, is a polyphenolic phytoalexin that is found in many natural plants and fruits, including grapes, pomegranates, and peanuts [7]. Resveratrol has beneficial effects on human health, including protective effects in cardiovascular disease, reduction in serum levels of low-density lipoprotein (LDL), and inhibition of platelet aggregation [8]. Resveratrol has also been proposed as a promising therapy for chronic kidney disease [9]. Saldanha et al. reported that resveratrol reduced renal damage by reducing inflammation and oxidative stress [10]. However, few studies have been conducted the investigate the molecular mechanisms for the renal effects of resveratrol.

Heat shock proteins were first identified following heat-associated stress of cells and tissues and were shown to modify the structure of other proteins [11]. Heat shock proteins have been identified as diagnostic, prognostic, and therapeutic biomarkers in human cancer [11]. Heat shock protein 70 (Hsp70) is the most structurally and functionally conserved protein in this family of proteins [12]. Hsp70 is a highly conserved functional protein across species [13]. In chronic kidney disease, the expression of Hsp70 is reported to be increased [14].

Lebherz-Eichinger et al. showed that Hsp70 was significantly increased in chronic kidney disease [15]. In patients with acute kidney injury associated with sepsis, survival was significantly worse in patients with higher clearance rates of Hsp70 and high mobility group box 1 (HMGB1) than the cutoff value [16]. In experimental models of sepsis, serum Hsp70 levels were significantly increased, and curcumin treatment reduced Hsp70 production [17]. MKT-077, a rhodacyanine dye, is a specific inhibitor of Hsp70, which was previously shown to have a role in human cancer [18].

Therefore, this study aimed to investigate the effects of resveratrol on kidney function in a rat model of uremia and the expression of heat shock proteins and mRNA, including Hsp70, and the effects of MKT-077.

Material and Methods

Animals

Three-month-old healthy specific pathogen-free (SPF) male Sprague-Dawley rats (N=163) with a mean weight of 220±30 g were purchased from Jinan Pengyue Experimental Animal Breeding Co., Ltd. The animals were studied under animal license number SCXK 2014-0007. The rats were maintained in an SPF class animal room laboratory with a room temperature of 22–24°C and relative humidity of 50–60% that was well-ventilated and quiet. The rats were free to drink and eat. Animal experiments were conducted according to the National Institutes of Health (NIH) revised 1996 Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23), and were approved by the Animal Protection and Use Committee of Yantaishan Hospital.

Establishment of the rat model of uremia and study groups

A rat model of uremia was established by 5/6 nephrectomy. Rats in each group fasted for 12 hours before surgery and were anesthetized by intraperitoneal injection of 3% sodium pentobarbital (50 mg/kg). The rats were in the prone position, and the skin of the surgical site was disinfected. An incision was made 1 cm below the left posterior rib and 0.5 cm from the spine to the subcutaneous muscles. The skin layers and subcutaneous tissues were cut to expose the left kidney. The left posterior rib was cut into 1 cm from the spine, the muscles were incised, the renal capsule was removed, and 2/3 of the lower part of the left kidney was removed. A gelatin sponge was used to control the bleeding, followed by suturing. After one week, anesthesia was repeated, and the same surgical procedure was performed on the right side, The right kidney was removed after ligation of the renal pedicle at the right renal hilum. Penicillin was given for three days to prevent infection following the left and right nephrectomy.

The effects of resveratrol on the kidney and the expression of heat-shock proteins in the rat model of uremic kidney injury were studied 40 rats in the model. The rats were randomly divided into four groups. The sham surgery group underwent opening of the renal capsule to expose the kidney for 5 minutes, followed by suturing of the incision (n=10). The model group developed uremia following 5/6 nephrectomy followed by intravenous saline administration for 28 days (n=10). The solvent-treated control group at one week following the creation of the model was treated with intravenous 0.5% hydroxymethyl cellulose sodium for 28 days (n=10). The resveratrol (Res) group included rats in the model of uremia that were given 20 mg/kg of oral resveratrol (suspended in 0.5% hydroxymethyl cellulose sodium) per day for 28 days (n=10).

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Table 1. The primer sequences used in this study.

Gene	Primer sequence
HSP70	Forward: 5'-GGACATCAGCCAGAACAAGC-3' Reverse: 5'-CCCTCGAACAGAGTCGAT-3'
HSP90	Forward: 5'-CTCTTCCTCCGCTCTTTGGG-3' Reverse: 5'-GTCTAGTTGACCGTTCCGCA-3'
HSP27	Forward: 5'-AAGTTTCCTCCTCCCTGTCC-3' Reverse: 5'-CATCGGATTTTGCAGCTTCT-3'
HSP25	Forward: 5'- GGACACTTTCCACATTGCTG-3' Reverse: 5'- CACTGTCTAGAAAAGACCCC-3'
HSP40	Forward: 5'- ATTCCCGTCGTGTTCAAAG-3' Reverse: 5'-ACCTGATGCCCTATTACTC-3'
HSP60	Forward: 5'-TGGGTCAAGTACTTTTATCCCCTA-3' Reverse: 5'-GGGAAGGCTAAGACCTACTCATT-3'
GAPDH	Forward: 5'-AGAAGGCTGGGGCTCATTTG -3' Reverse: 5'-AGGGGCCATCCACAGTCTTC -3'

Kidney histology

Twenty-four hours after treatment with resveratrol, the rats were anesthetized with an intraperitoneal injection of 3% sodium pentobarbital (50 mg/kg) and euthanized by cervical dislocation. Kidney tissues were sampled and fixed in a 4% paraformaldehyde solution. Tissues were dehydrated, embedded in paraffin wax, and sectioned onto glass slides at a thickness of 5 μm. The renal tissue sections were then dried, dewaxed, and hydrated in ethanol and stained with hematoxylin and eosin (H&E) (Solarbio, Beijing, China) for 5 min, rinsed with tap water, and mounted with glass coverslips. Histology was performed at a magnification of ×400 using a BX51 Olympus light microscope (Olympus, Tokyo, Japan). According to the degree of tubular necrosis, cell swelling, vacuolation, and exfoliation of the renal tubular epithelial cells, a five-point quantitative histological scoring method was used [19], as follows: 0, <10%; 1, 10–25%; 2, 25–50%; 3, 50–75%; 4, 75–100%.

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

The tissue samples were lysed and centrifuged at 10,000 rpm for 10 min. Total RNA was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA). The PrimeScript™ RT Reagent Kit (Takara, Minato-ku, Tokyo, Japan) was used to transcribe RNA into cDNA. The qRT-PCR was performed using SYBR Premix Ex Taq™ II (Takara, Minato-ku, Tokyo, Japan) at 95°C for 10 min, 95°C for 15 s, 60°C for 60 s (40 cycles). Data were processed by the $2^{-\Delta\Delta Ct}$ method. The relative expression levels were calculated using GAPDH mRNA as an internal reference. The primers used in this study (Shanghai Shenggong Bioengineering Technology Service Co., Ltd., Shanghai, China) are shown in Table 1.

Western blot

Total protein was extracted from the tissues, and the protein concentration was measured using a Pierce BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Then, 40 μg of each sample was loaded and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (Bio-Rad, Hercules, CA, USA). After separation, the proteins were transferred onto a polyvinylidene fluoride (PVDF) membrane (Merck Millipore, Burlington, MA, USA), and blocked with 5% dried skimmed milk powder for one hour. The membranes were incubated with the primary rabbit polyclonal antibodies to Hsp70 (1: 10000) (ab79852; Abcam, Cambridge, UK) and β-actin (1: 1000) (ab8227; Abcam, Cambridge, UK) overnight at 4°C. The membrane was washed three times using TBST, containing TBS and 1 ml/L of Tween-20, for 10 mins. The membranes were then incubated with goat anti-rabbit IgG (1: 2000) (ab6721; Abcam, Cambridge, UK) for one hour at room temperature. After washing, the results were detected using enhanced chemiluminescence (ECL) and quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Protein expression levels were normalized to β-actin.

The study groups using the rat model and the use of the Hsp70 inhibitor, MKT-077

Using the uremic rat model, 120 healthy SPF male Sprague-Dawley rats were randomly divided into six groups. The sham operation group (sham group) underwent opening of the renal capsule, and the muscle layer and skin were sutured after 5 minutes. The model group that underwent 5/6 nephrectomy, was given intravenous saline for 28 days after modeling. The model group was treated with the Hsp70 inhibitor, MKT-077, after one week of modeling, using 0.75 mg/kg of MKT-077 that was intravenously administered every day for 28 days. The solvent-treated control group, after one week of modeling, were given oral 0.5% hydroxy methylcellulose sodium daily for 28 days. The resveratrol group (Res group), one week after the establishment of the model, was given 20 mg/kg of oral resveratrol per day (suspended in 0.5% hydroxymethyl cellulose sodium) for 28 days. The resveratrol and Hsp70 inhibitor group (Res+MKT-077 group), one week after the establishment of the model, was given 20 mg/kg of oral resveratrol per day, with an intravenous injection of 0.75 mg/kg MKT-077 for 28 days. Figure 1 shows the animal groups and treatment protocol used.

Outcome indicators

The survival rate in the rats after modeling was assessed on a weekly basis and analyzed using survival curves. Renal function was evaluated six weeks after modeling, by blood sampling from the rat tail vein, followed by centrifuging the blood at 3,000 rpm for 10 min. Blood urea nitrogen (BUN) and serum

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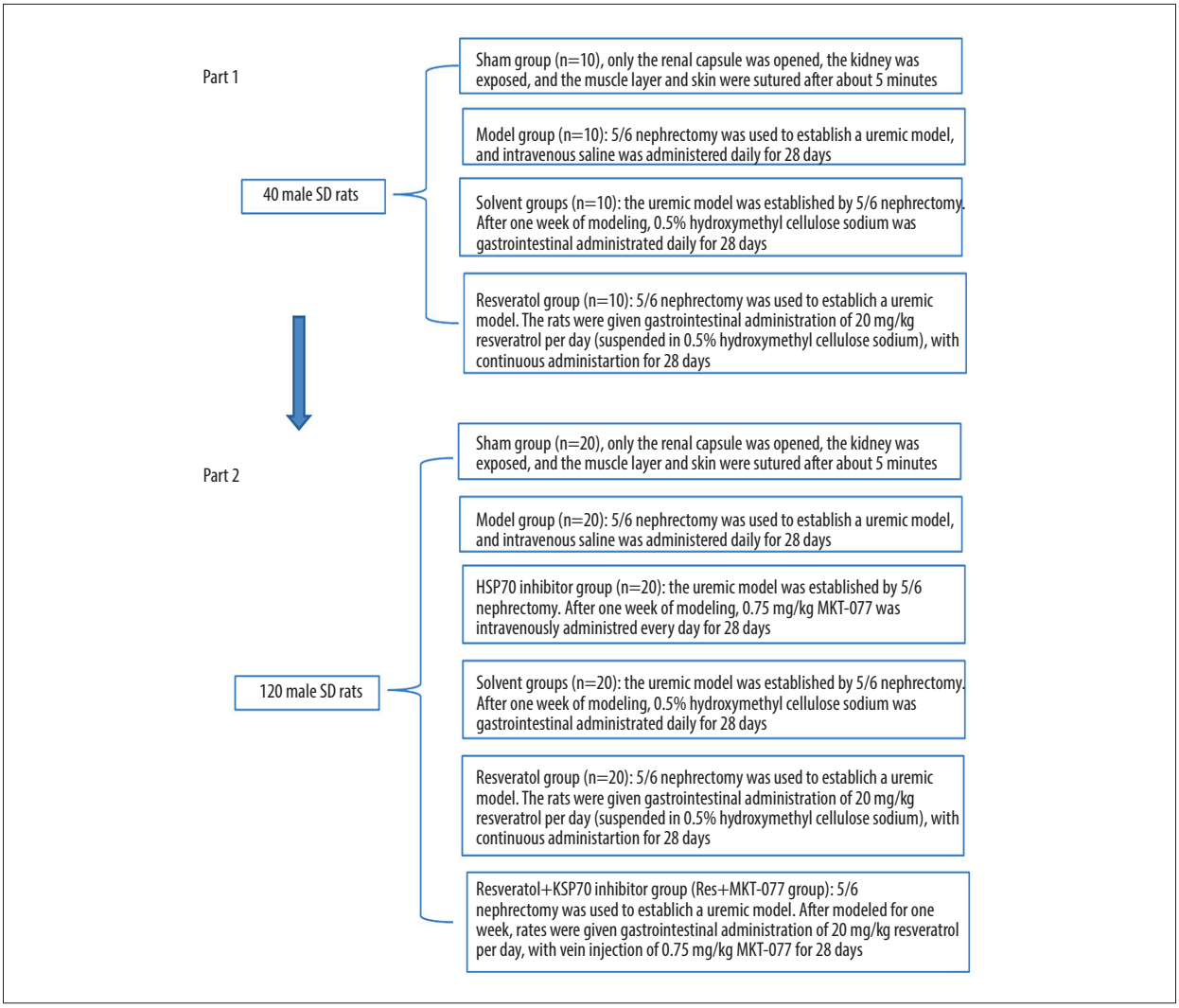

Figure 1. Diagram of the study design.

creatinine levels were determined using a standard diagnostic kit (Span Diagnostics, Gujarat, India). Urine specimens (5mL) were collected for 24 hours using a metabolic cage and centrifuged at 3000 rpm for 3 min, and the supernatant was taken. The urine protein content was determined using an automatic biochemical analyzer. After six weeks, six surviving rats were randomly selected from each group. After anesthesia with 3% sodium pentobarbital (50 mg/kg), the rats were euthanized by cervical dislocation and the kidney tissue was sampled. Kidney tissue was fixed in 4% paraformaldehyde solution for histology, TUNEL, and immunohistochemistry. Some kidney tissue samples were stored in liquid nitrogen at –80°C for quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis.

Detection of apoptosis in renal tissue by the TUNEL assay

Renal tissues were fixed in 4% paraformaldehyde solution, embedded in paraffin wax, and sectioned onto glass slides at 4 μm. Tissue sections were dewaxed in xylene and dehydrated in graded ethanol. Cell apoptosis was quantified by the TUNEL assay using a ZK-8005 apoptosis detection kit (Beijing Zhongshan Jinqiao Biotechnology Co., Ltd., China). Five fields were randomly selected by light microscopy to observe the cell apoptosis at ×400 magnification.

Detection of P53, BAX, and BCL-2 mRNA by quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed for 40 cycles of 95°C for 10 min, 95°C for 15 s, and 60°C for 60 s. Data were processed by the $2^{-\Delta\Delta Ct}$ method. The relative expression levels were calculated

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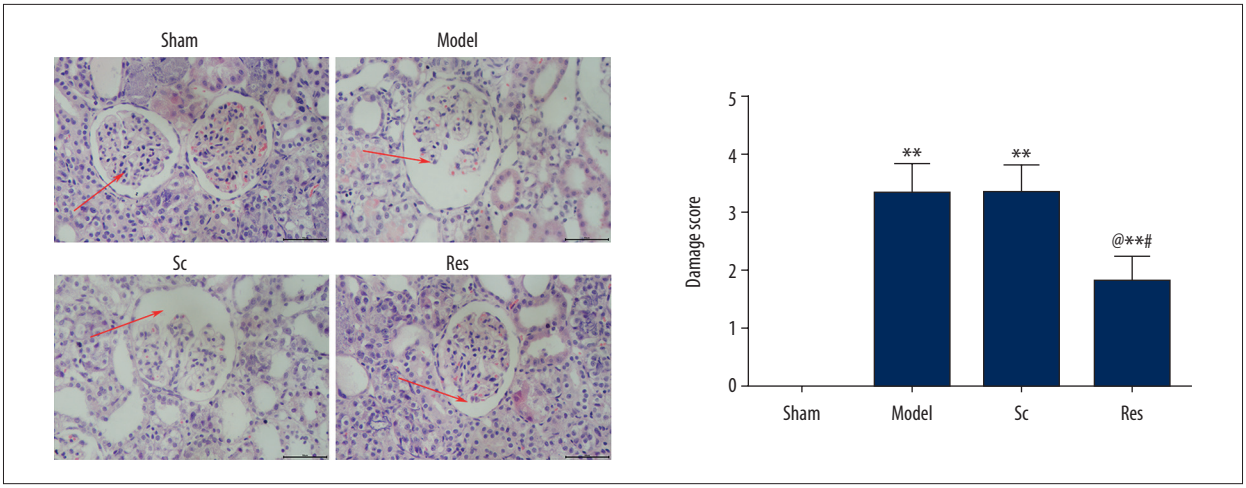

Figure 2. The histology of the kidney in the rat study groups using hematoxylin and eosin (H&E) staining and a magnification of ×400. The red arrow indicates the renal glomerulus. The study groups (N=5) included: the Sham group that underwent sham surgery; the Model group, the untreated rat model of uremia; the Sc group, the solvent-treated control group; and the resveratrol group, treated with resveratrol. Data are presented as the mean±standard deviation (SD). Comparisons *vs.* the sham group, ** p<0.01; *vs.* the Model group, # p<0.05; *vs.* the solvent-treated control (Sc) group, @ p<0.05.

using GAPDH mRNA as an internal reference. The following primers were used:
P53, forward: 5'-TCACAGCGTCTGTTGACATTT-3';
P53, reverse: 5'-ACCAAGCTCATTACCCTGACA -3';
BAX, forward: 5'- ATCTGGTTCTGCAAGCGTTTA-3';
BAX, reverse: 5'-CCTGCTCCGAATTTG GTGAAA-3';
BCL-2, forward: 5'- ATGTGTGTGGAGAGCGTCAA-3';
BCL-2, reverse: 5'-ACAGTTCC ACAAAGGCATCC-3';
GAPDH, forward: 5'-AGAAGGCTGGGGCTCATTTG-3';
GAPDH, reverse: 5'-AGGGGCCATCCACAGTCTTC-3'.

Immunohistochemical detection of p53, Bax, and Bcl-2 in rat kidney tissue

The kidney tissue sections that had been fixed in 4% paraformaldehyde solution, paraffin-embedded, and sectioned onto glass slides were dewaxed and stained with primary antibodies using a streptavidin-biotin complex (SABC) immunohistochemistry detection kit. Immunostaining was localized with the brown chromogen, 3,3'-diaminobenzidine (DAB). The tissue sections were evaluated by light microscopy at a magnification of ×400.

Statistical analysis

Data were analyzed using GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA, USA). Data were expressed as the mean±standard deviation (SD). The data was analysis between multiple groups using one-way analysis of variance (ANOVA), and the subsequent analysis was performed by Tukey's post hoc test. Survival curves were developed using GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA, USA) and analyzed by the log-rank test. A P-value <0.05 was considered to be statistically significant.

Results

Resveratrol treatment reduced renal tissue damage in the rat model of uremia

Figure 2 shows the histological changes in the rat kidney tissue. Rats in the sham group showed normal glomerular and renal interstitial histology. The rat model of uremia following 5/6 nephrectomy showed significant glomerular changes and damage of the tubular epithelium with interstitial inflammation (p<0.05). Histology of the renal tissue in the solvent-treated control group was similar to that of the model group. Compared with the model group, resveratrol treatment significantly reduced the renal tissue changes, reduced the degree of inflammation, and the changes in the renal glomeruli (p<0.05).

Resveratrol treatment increased the expression of HSP70 mRNA in renal tissue of uremic rats

Figure 3 shows that when compared with the sham group, the mRNA expression of HSP70, HSP90, HSP27, HSP25, HSP40, and HSP60 mRNA in the kidney of the model group were all significantly increased (Figure 3A). HSP70 mRNA in the resveratrol group was significantly increased when compared with the model group (p<0.05). The expression of HSP70 was detected by Western blot (Figure 3B). These results showed that the expression of HSP70 protein in the resveratrol group was significantly greater than that in the other groups (p<0.05).

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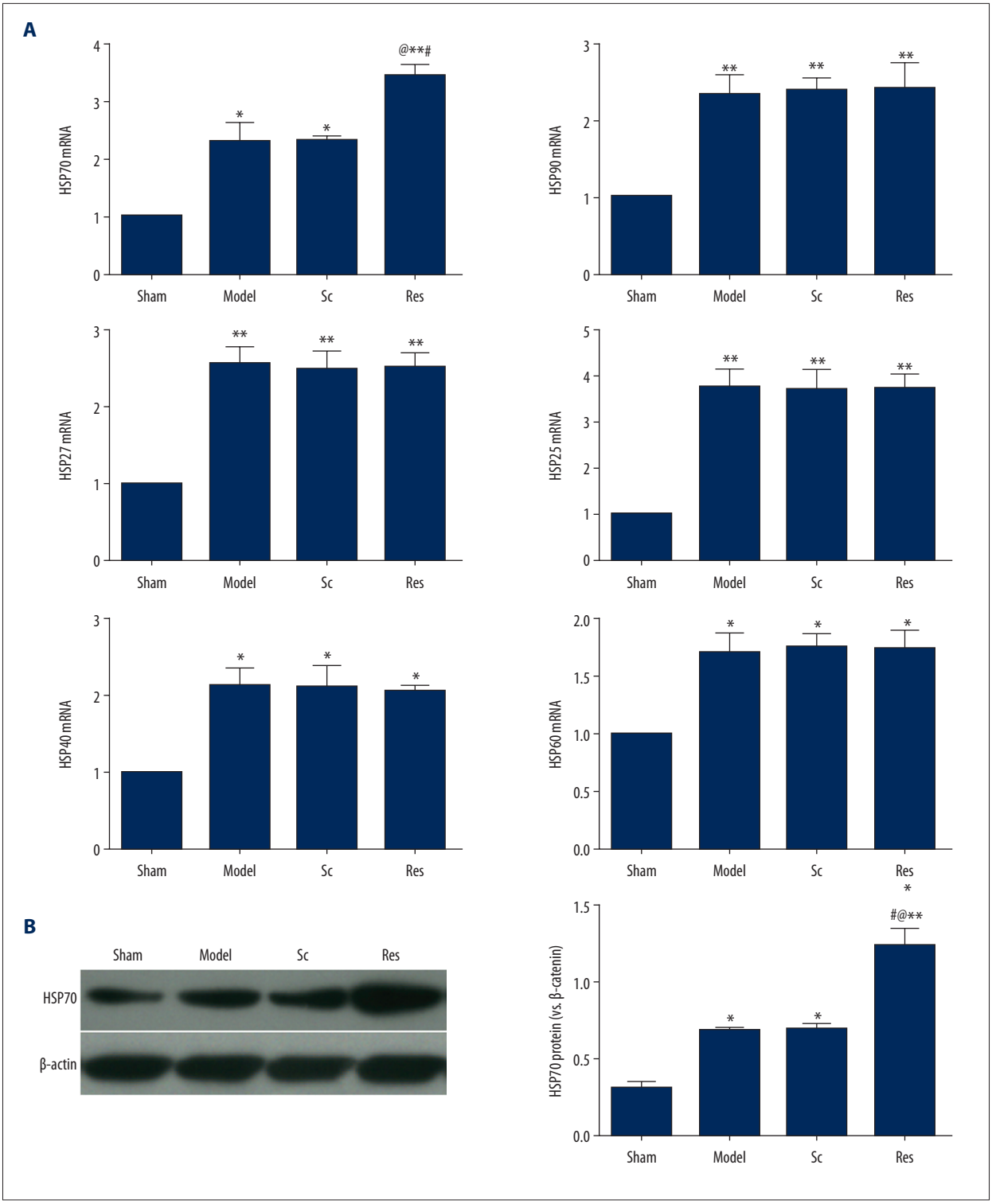

Figure 3. Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and immunohistochemistry for the detection of mRNA and protein levels in rat renal tissue. The mRNA expression of HSP70, HSP90, HSP27, HSP25, HSP40, and HSP60 in renal tissue of uremic rats (N=5) after treatment with resveratrol is shown (**A**). Western blot detected the expression of Hsp70 in rat kidney after treatment with resveratrol (**B**). Data are presented as the mean±standard deviation (SD). Comparisons *vs.* the sham group, ** p<0.01; *vs.* the Model group, # p<0.05; *vs.* the solvent-treated control (Sc) group, @ p<0.05.

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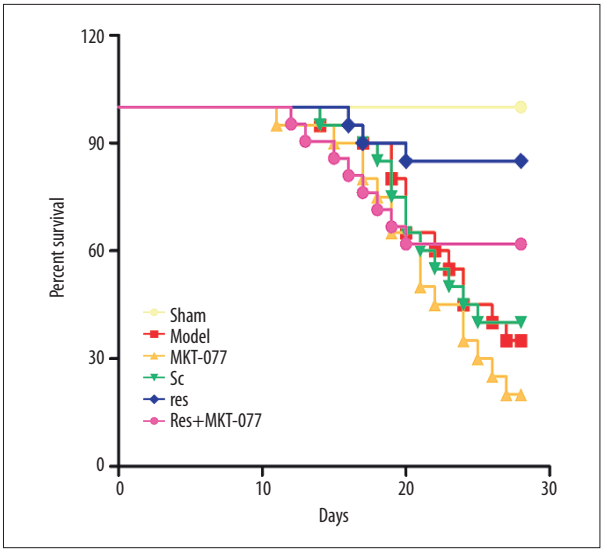

Figure 4. The survival rates of the rats in the different study groups. Resveratrol treatment increased the survival rate through the regulation of Hsp70 in uremic rats. In each group, there were 20 rats. MKT-007, Hsp70 inhibitor group (MKT-077); Res+MKT-007: resveratrol+Hsp70 inhibitor group.

Resveratrol increased survival rate in uremic rats by increasing Hsp70 expression

After one week of modeling, the rats in each group, except the sham group, had a different prevalence in mortality (Figure 4). After six weeks, the statistical results showed that the survival rate of rats in the sham group was 100%. The survival rate of the model group was 35%, which was significantly lower than the sham group. Compared with the model group, the survival rate of the MKT-077 group decreased to 20%, and the survival rate of the solvent-treated control group was reduced to 40%. After resveratrol treatment, the numbers of death in the resveratrol group were reduced to 85%. Compared with the resveratrol group, the survival rate of the resveratrol+MKT-077 group was 60%.

Resveratrol improved renal function in uremic rats by increasing HSP70 expression

Figure 5 shows that compared with the sham group, the contents of serum creatinine, blood urea nitrogen (BUN), and urine protein in the model group were significantly increased (p<0.01). Compared with the model group, the value of the three

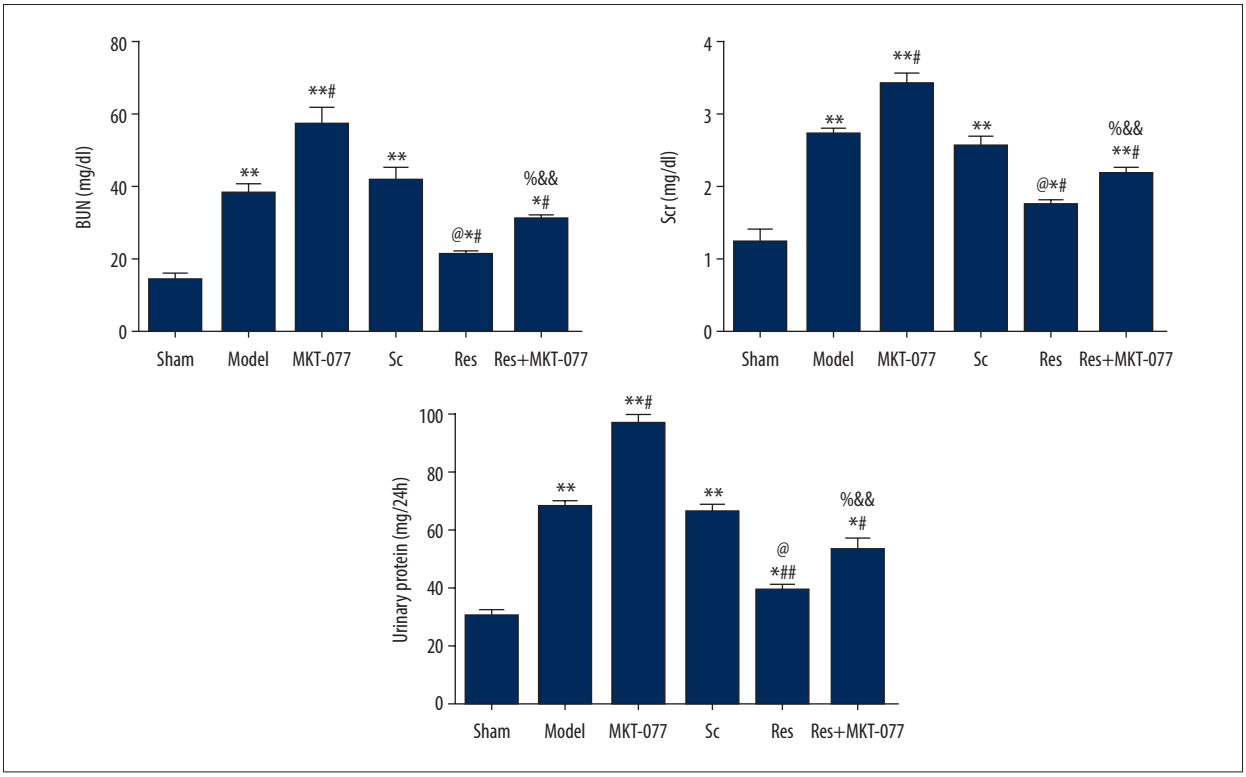

Figure 5. Resveratrol reduced the expression of blood urea nitrogen (BUN), serum creatinine, and urinary protein through regulation of Hsp70 in the rat model of uremia. Data are presented as the mean ± standard deviation (SD). In each group, the number of rats was 5. * p<0.05, ** p<0.01 compared with the sham group; # p<0.05, ## p<0.01 compared with the model group; @ p<0.05 compared with the solvent-treated control group;%p<0.05 compared with the resveratrol group. && p<0.01 compared with the MKT-077 group.

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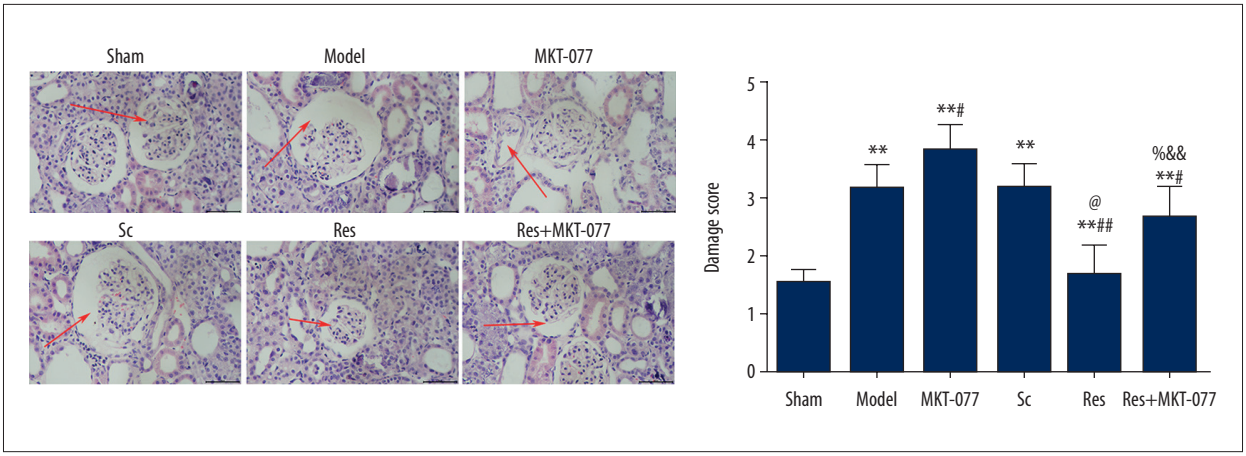

Figure 6. The histology of the kidney in the rat study groups using hematoxylin and eosin (H&E) staining and a magnification of ×400. The red arrow indicates the renal glomerulus. The study groups (N=5) included: the Sham group that underwent sham surgery; the Model group, the untreated rat model of uremia; the Sc group, the solvent-treated control group; and the Res group, treated with resveratrol. Data are presented as the mean±standard deviation (SD). There were 5 rats in each group. * p<0.05, ** p<0.01 compared with the Sham group; # p<0.05, ## p<0.01 compared with the Model group; @ p<0.05 compared with the solvent-treated control (Sc) group; % p<0.05 compared with the resveratrol group; && p<0.01 compared with MKT-077 group.

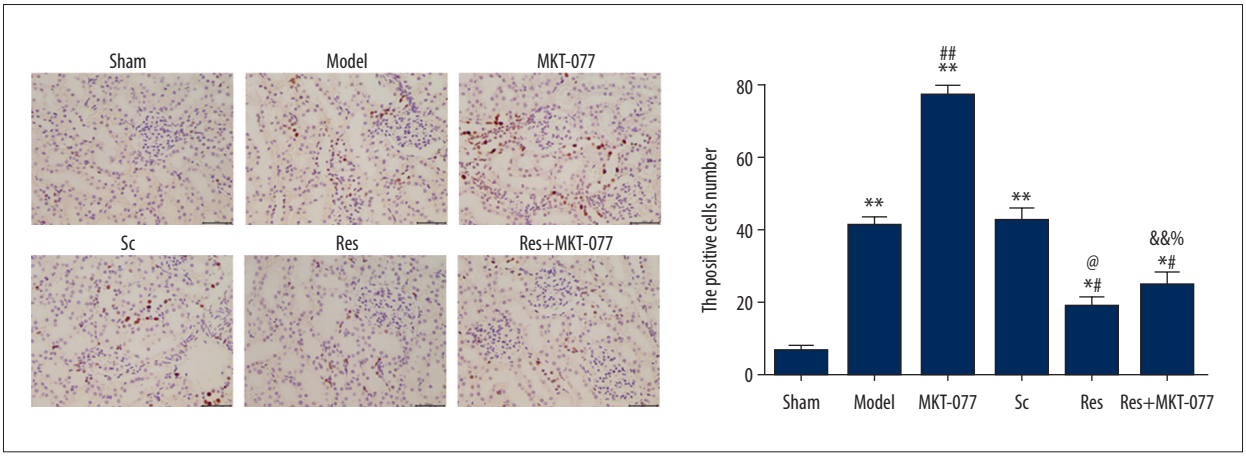

Figure 7. Apoptosis in the kidney in the rat model of uremia shown by TUNEL at a magnification of ×400. Resveratrol reduced renal cell apoptosis in uremic rats by regulating the expression of Hsp70. Data are presented as the mean±standard deviation (SD). In each group, the number of rats was 5. * p<0.05, ** p<0.01 compared with the sham group; # p<0.05, ## p<0.01 compared with the model group; @ p<0.05 compared with the solvent-treated control group; % p<0.05 compared with the resveratrol group; && p<0.01 compared with MKT-077 group.

indicators in the MKT-077 group increased significantly (p<0.05), but there was no significant difference in the three indicators between the model and the solvent-treated control group. Resveratrol treatment significantly reduced the three indicators in the rats, while treatment with MKT-077 significantly reversed the effect of resveratrol when compared with the resveratrol group (p<0.05).

Resveratrol reduced renal tissue damage in uremic rats by increasing Hsp70 expression

Figure 6 shows the results of the histology of the rat kidneys. The renal tissue morphology of the sham group was normal. The glomerular wall of rats in the model group was thickened, and there was a significant inflammatory cell infiltrate. Compared with the model group, the MKT-077 group had increased glomerular damage (p<0.05). The renal tissue morphology of the solvent-treated control group was similar to that of the model group, while resveratrol treatment significantly

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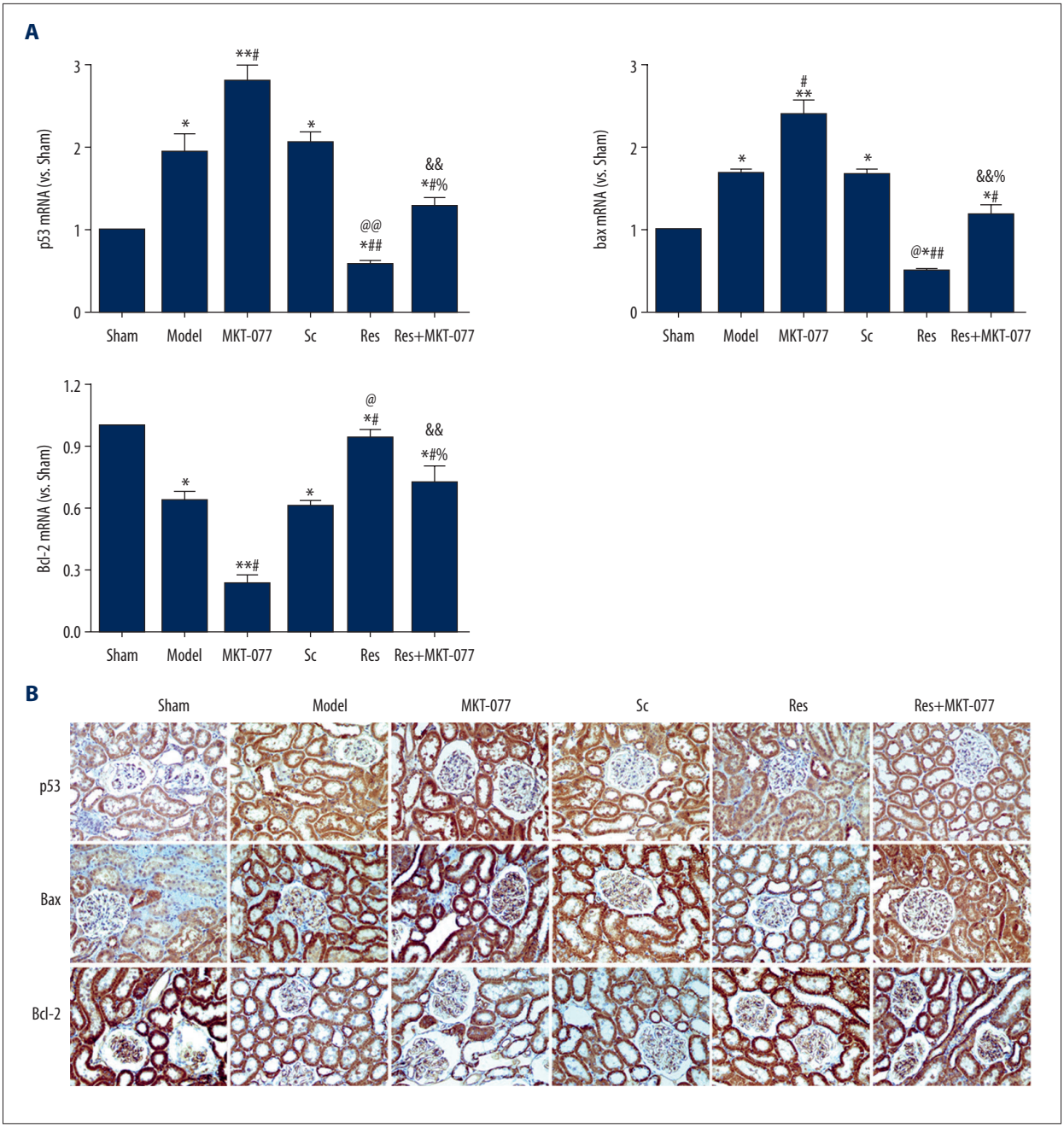

Figure 8. The expression of P53, BAX, and BCL-2 mRNA was determined by quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and immunohistochemistry. Resveratrol regulated the expression of HSP70 mRNA and Hsp70 protein expression and the expression of P53, BAX, and BCL-2 in the renal tissue of rats in the model of uremia. (**A**) Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) detection of P53, BAX, and BCL-2 mRNA expression levels in rat kidney tissue. (**B**) Immunohistochemical staining of rat kidney tissue. Magnification ×400. Data are presented as the mean ± standard deviation (SD). Compared with the sham group, * $p<0.05$, ** $p<0.01$; compared with the model group, # $p<0.05$, ## $p<0.01$; compared with the solvent-treated control (Sc) group, @ $p<0.05$, @@ $p<0.01$; compared with the resveratrol group, % $p<0.05$; compared with MKT-077 group, && $p<0.01$.

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reduced the renal tissue lesions and reduced the inflammatory response, glomerular changes ($p<0.05$). However, the renal tissue lesions of the resveratrol+MKT-077 group were significantly more severe than those of the MKT-077 group, which reversed the positive effects of resveratrol ($p<0.01$).

Resveratrol reduced renal cell apoptosis in uremic rats by increasing Hsp70 expression

The results of the TUNEL assay for cell apoptosis are shown in Figure 7. Compared with the sham group, apoptosis of renal cells in the model group was significantly increased ($p<0.01$). Compared with the model group, the number of apoptotic cells in the MKT-077 group increased ($p<0.01$), and the number of apoptotic cells in the resveratrol group was significantly reduced ($p<0.05$). Compared with the MKT-007 group, the number of apoptotic cells in the resveratrol + MKT-077 group was significantly increased ($p<0.01$).

Resveratrol affected P53, BAX and BCL-2 mRNA and protein expression in renal tissues of uremic rats by increasing Hsp70 expression

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) and immunohistochemistry were used to detect the expression levels of P53, BAX and BCL-2 mRNA (Figure 8A) and protein (Figure 8B) in the rat kidney tissues. Compared with the sham group, the levels of P53 and BAX in the kidney of the model group were significantly increased ($p<0.05$), while BCL-2 expression was reduced. Compared with the model group, the expression of P53 and BAX was further increased, and the expression of BCL-2 was reduced in the MKT-007 group ($p<0.05$). The expression level of each protein in the solvent-treated control group was similar to that in the model group. After resveratrol treatment, the expression of P53 and BAX was significantly increased, and the expression of BCL-2 was significantly reduced ($p<0.05$). Compared with the MKT-007 group, the expression of P53 and BAX increased, and BCL-2 expression was reduced in the resveratrol+MKT-077 group ($p<0.05$).

Discussion

The aim of this study was to investigate the effects of resveratrol on kidney function in a rat model of uremia and the expression of heat shock proteins (HSPs). The findings showed that resveratrol treatment significantly improved renal tissue lesions in rats. Also, resveratrol could improve renal tissue damage and reduced renal cell apoptosis in uremic rats by increasing Hsp70 expression. Hsp70 has been previously reported to result in cellular protection against biological stress *in vitro* and *in vivo* [20–23]. Resveratrol induces a heat-shock response and protects human cells from heat stress [24,25]. Chakraborty et al. showed that resveratrol could accelerate the apoptosis of chronic myelogenous leukemia (CML) cells by targeting heat shock protein 70 (Hsp70) [26]. The findings from the present study showed that the mRNA expression of HSP70, HSP90, HSP27, HSP25, HSP40, and HSP60 in the kidney of the rat model of uremia were increased compared with the sham group. Also, protein expression of Hsp70 after resveratrol treatment was significantly increased when compared with the sham group and the untreated model group. These data support that resveratrol increased the level of Hsp70 in renal tissues, which is supported by findings from previous studies. In 2005, Nakada et al. reported that the administration of resveratrol followed by Hsp70 induction could improve the survival rate in a rat model of endotoxin shock [27]. The findings from the present study showed that the survival rate of the resveratrol-treated rats in the uremia model increased significantly compared with the untreated model group.

Increased expressions of Hsp70 has previously been reported to have a beneficial role in disease processes. In 2000, Bidmon et al. showed that Hsp70 expression was associated with repair of the proximal tubule following renal ischemia [28]. The findings from the present study showed that resveratrol treatment significantly reduced serum creatinine, blood urea nitrogen (BUN), and urine protein levels, while resveratrol treatment with the Hsp70 inhibitor, MKT-077, significantly reversed the effects of resveratrol ($p<0.05$). The findings from the present study supported that resveratrol improved renal function in uremic rats by increasing Hsp70 expression.

Hsp70 expression has previously been shown to have beneficial effect on renal injury through the inhibition of inflammation and reduced expression of inflammatory factors [29]. High expression levels of Hsp70 modify proinflammatory and pro-apoptotic mediators and reduce cell apoptosis. However, the renal tissue lesions of the resveratrol + MKT-077 group were worse than those of the resveratrol group, which supported that resveratrol could reduce renal tissue damage and reduce renal cell apoptosis in uremic rats by increasing Hsp70 expression.

Conclusions

This study aimed to investigate the effects of resveratrol on kidney function in a rat model of uremia and the expression of heat shock proteins. In the rat model of uremia, resveratrol reduced renal injury and improved both renal function and survival, which were associated with increased expression of Hsp70.

Conflict of interest

None.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

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