Establishment of a Novel in Vivo Rabbit Model for Hypothermic Machine Perfusion

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Method Article

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Abstract

This study aims to establish a novel animal model and lay the foundation for the research on the mechanism of hypothermic machine perfusion \(MP). Based on different left renal warm ischemia 25min, 35min and 45min, they are grouped into MP 25min, cold storage \(CS) 25min, MP 35min, CS 35min, MP 45min and CS45min \(n=5), followed by reperfusion for 1 h. Perform in vivo hypothermic preservation \(MP or CS) for 4 hrs. After 24 hrs reperfusion, the specimens are collected. Results showed that postoperative 24-hour survival rates of 25 min group were 100%, while survival time of 45min group were 0% \(p>0.05). In conclusion, an in vivo rabbit model for MP is a novel, effective and stable animal model. MP improves kidney viability in rabbits below 35 minutes' warm ischemia, which is invalid after 45 minutes' warm ischemia.

Introduction

Organ transplantation has become the effective treatment for end-stage renal disease \[1].Since DCD was approved by WHO \[2], DCD has become an increasingly used source internationally for the past decade \[3-5]. Nevertheless, compared to DBD donor, DCD has some shortcomings \[3]. For example, the warm ischemia time of DCD donor organ is longer, which will lead to high incidence of primary nonfunction \(PNF) or delayed graft function \(DGF) after operation \[6]. At the same time, there are many problems during the process of organ transplantation. Despite of great improvement, the method of organ preservation still remains a key and urgent problem that needs to be solved for transplantation \[7]. Therefore, organ preservation method needs further clinical optimization to reduce organ damage \[8]. Currently the methods commonly used are MP and CS, but so far there lacks specific clinical indicators or stipulations on how to choose MP or CS. Many studies show that the application of hypothermic machine perfusion has better effect than cold preservation \[9, 15, 16], especially in renal transplantation because the perfusion liquid can be injected into the kidney from the renal artery by hypothermic machine perfusion, which can scour the vascular and protect the vascular bed \[10]. Meanwhile, MP can reduce the incidence of DGF \[11-14], and avoid the shortcomings of CS method \[17]. Mechanism of hypothermic machine perfusion improving DCD donor renal activity has not been fully understood \[18], and therefore further study is urgently required; however, a stable and reliable animal model with high repeatability is not yet available clinically. With reference to the whole process of clinical renal transplantation, we wonder whether the whole process of DCD renal transplantation ischemia reperfusion can be simulated in the body of rabbits, and this becomes the core technology we focus on. Therefore, we have innovatively established an in vivo model for hypothermic machine perfusion in rabbits to study the mechanism of hypothermic machine perfusion. It will provide a basis for similar studies on the mechanism of machine perfusion and clinical translational medical research in the future. Because of the new model, further mechanism had been illuminated \[19-23].

Reagents

\(1) 36 healthy, male rabbits\(Wuhan Wanqianjiahe Experimental Animal Breeding Center, China, 12-16 weeks old, body weight of 3.5±0.3kg)\!CAUTION: All animal experiments must comply with national laws and institutional regulations \(2) 0.9% Sodium Chloride Solution \(Wuhan Binhu Double-crane Pharmaceutical Co.,Ltd., China, GUOYAOZHUNZI H42020474, Batch No.:1410070301) \(3) 5% Glucose and Sodium Chloride Injection \(No. 4 Pharmaceutical Co., Ltd., Shijiazhang, China, GUOYAOZHUNZI H13022490, Batch No.: 1407301403) \(4) 1%Povidonelodine \(Wuhan Yunzuo Fine Chemical Co., Ltd., China) \(5) Pentobarbital \(Merck Drugs & Biotechnology, Germany) \(6) Ceftazidime \(Hainan Hailing Chemipharma Corporation Limited, China, GUOYAOZHUNZI H20023524, Batch No.:1409053) \(7) Lactated Ringer Solution \(No. 4 Pharmaceutical Co., Ltd., China, GUOYAOZHUNZI H20044961, Batch No.:1404221703) \(8) Furosemide \(South Land Pharmaceutical Co., Ltd., China, GUOYAOZHUNZI H44022506, Batch No.:1408131) \(9) Calcium Gluconate \(Anyang Qiuzhou Pharmaceutical Co., Ltd., China, GUOYAOZHUNZI H41023479, Batch No.:1407660260) \(10) Sodium Bicarbonate Injection \(China Huiyinbi Group East Asia Pharmaceutical Co., Ltd., GUOYAOZHUNZI H36020283, Batch No.:2014092915) \(11) Hydroxyethyl Starch Injection \(Nanjing Chia Tai Tianqing Pharmaceutical Co., Ltd., China, GUOYAOZHUNZI H20065430, Batch No.:1409253) \(12) Hypertonic Citrate Adenine Solution for Isolated Kidney Preservation \(Shanghai Changzheng Hospital, China) \(13) Dopamine HCl\(Guangzhou Baiyun Shan Ming Xing Pharmaceutical Co., Ltd., China) \(14) Heparin \(Wanbang Pharmaceutical Co., Ltd., China, Batch No.:1407102)

Equipment

Apparatus and appliance \(1) Disposable mask \(Xinxiang City Kangmin Hygienic Supply Development Co., Ltd., China \(2) Cotton pads, gauze, cotton ball \(Xinxiang City Kangmin Hygienic Supply Development Co., Ltd., China) \(3) Disposable surgical drape, disposable surgical gowns \(Xinxiang City Kangmin Hygienic Supply Development Co., Ltd., China) \(4) 1ml, 2.5ml, 5ml, 10ml, 20ml syringe \(Jiangxi Hongda Medical Equipment Group, China) \(5) Scalp Acupuncture \(Jiangsu Suyun Medical Material Co., Ltd., China) \(6) Applicator \(Wubei Wuhu Medical Equipment Group Co., Ltd., China) \(7) Adhesive plaster \(China Minnesota Mining Manufacturing and Medical Supplies Co., Ltd.) \(8) Suture thread \(Johnson & Johnson Medical Equipment \(China) Co., Ltd.) Caution: 3-0 is used for suturing the skin, while 4-0 is used for ligating the blood vessels. \(9) Suture needle \(Shanghai PudongJinhuan Medical Products Co., Ltd., China) Caution: three-edged needle is used for suturing the skin, while round needle is used for suturing the muscles and connective tissues. \(10) Micro Instruments \(Ningbo Cheng-He Microsurgical Instruments Factory, China) \(11) Suture needle with line \(Ningbo Medical Needle Co., Ltd., China, Batch No.: 130603) \(12) Bloodgas analyzer \(Abbott Point of Care inc. IL 60064, USA, SN 2-52661) \(13) Lifeport Perfusion apparatus \(Organ Recovery systems, IL, USA, SN LKT 1233508, REF LKT 100P) \(14) Surgical Instruments \(Shanghai Medical Instruments \(Group) Ltd., Corp. Surgical Instrument Factory) \(15) JR-1/2Intelligent Temperature Control Instrument \(Chengdu Taimeng Science and Technology Co., Ltd., China) \(16) Operating table \(XinghuaTongchang stainless steel factory, China) \(17) DK-A Animal High Frequency Electrotome \(Hefei Golden Brains Optical Instrument Co., Ltd., China)

Procedure

Experimental groups 30 healthy, adult New Zealand rabbits, male, weight \(3.5 ± 0.3) kg, 12-16 weeks old, were randomly divided into MP group and CS group. Based on different left renal warm ischemia 25min, 35min and 45min, the two groups are further grouped into MP 25min, CS 25 min, MP 35 min, CS 35 min, MP 45 min and CS 45 min, with 5 rabbits in each sub-group. **In vivo model establishment for hypothermic machine perfusion in rabbits** _Preoperative preparation_ Select two male rabbits \(body weight 3.2-3.8kg) each time, and remove their bunk feeders 36-48h before the operation. Make sure that they are fasted 36 to 48hrs but sufficient water should be guaranteed in case of accidental death. The surgical instruments sterilized under high heat and pressure should be placed in the tray spread with a disposable sterile towel prior to the operation. The heating panels and small animal electrotome should be fixed in advance. Then, turn on the air conditioning to maintain the operation room at 25°C/50-70% RH. Finally, switch on the shadowless surgical operating lamp. _Intraoperative operation_ \(1) Anesthetize and weigh the fasted male rabbits. The rabbits are anesthetized by injecting 1% sodium pentobarbital solution \(3ml/kg body weight) in the rabbit's right ear marginal vein through 20 ml syringe and scalp acupuncture. After the conjunctival reaction disappears or becomes dull, clamp reflex also disappears, the rabbits are all anesthetized with stable breathing and the body tension is reduced. Fix the scalp acupuncture, establish vein channel, and give intravenous injection of 5% glucose solution at 5s/drop. Maintain the rabbit's basal body temperature \(38±0.5°C). \(Supplementary photo 1) \(2) Fix the heating plate set at 45.0°C on the operation table. Place the rabbits on the heating plate in supine position with limbs and head fastened to keep breathing unobstructed. \(Supplementary photo 2) \(3) Skin preparation: prepare the skin between the xiphoid process and symphysis pubis for ventral midline celiotomy, and make sure sufficient skin of left abdomen and lumbar region are prepared. Prepare the left femoral skin, and paste the electrosurgical dispersive plate on it, meanwhile insert the rectal temperature probe into the anus only after defecation, otherwise the probe will bend, and temperature measurement will be not accurate. \(Supplementary photo 3) \(4) Wash hands according to the clinical sterile requirements, and wear the disposable aseptic surgical gowns. \(5) Soak the sterile gauze in povidone iodine, then disinfect the operation skin completely by imbricate method, and repeat this 3 times. \(Supplementary photo 4) \(6) Take a disposable aseptic hole-towel to drape the abdominal area, and make sure the surgical area is exposed. Cover with disposable surgical sterile towel, and cut in the middle to get a window hole so that the operation can be revealed from the hole. \(Supplementary photo 5) \(7) Connect the electrotome and suction apparatus, then carry out conventional laparotomy with abdominal incision. Gash the skin below the xiphoid process to 3-5cm above the pubic symphysis. If bleeding happens, stop it with pressing gauze and electric coagulation hemostasis, and ligation should be avoided as much as possible. Incise the muscle along the linea alba with the electrotome after hemostasis of skin. Attention should be given to cut muscle layer with slightly raising the muscle to protect the abdominal viscera from injury. More bleeding will happen in the muscle layer, and also liable to hemodiapedesis, electric coagulation hemostasis or incision suture method is often used until hemodiapedesis is completely stopped. \(Supplementary photo 6) \(8) Clamping the renal pedicle and abdominal exploration: protect the cut edge with a cotton pad and gauze, push slightly the abdominal

organs aside to the right, cover them with gauze, and keep the moisture and temperature with 45.0°C normal saline all the time to prevent intestinal necrosis and dehydration adhesion. Expose the left kidney, and free the left ureter. Blunt dissection of renal vein should be conducted gently to prevent injury and tear. During the blunt dissection of renal artery, whether there is artery malformation or multiple arteries should be determined, and make sure arterial sheath should be isolated well with the length of 1.5-2cm. After finishing the separation, firstly close the ureter by artery clamp, then close renal artery and vein at the same time, start timing from the moment of clipping. Observe the renal color and texture, the kidney should be darkened and harder, while beating of artery should be stopped. Clipping time is 25min/35min/45min. Clip simply the incision skin using tissue forceps to close the abdominal cavity, and maintain the basal body temperature at 38±0.5°C. Draw the blood from the vein of left ear, and carry out blood gas analysis. \(Supplementary photo 7) \(9) Release the tissue forceps 1 min before reaching 25min/35min/45min reperfusion, open the abdominal cavity to expose the left kidney, and release two arterial clips the moment reaching 25min/35min/45min. Observe the renal color and texture; the kidney should be seen reddened and softened without petechiae, and pulsation of artery should restore. Be cautious to protect the intestinal tract. Start timing. Simply clip the incision skin using tissue forceps to close the abdominal cavity, and maintain the basal body temperature. Reperfusion time is 1h. \(Supplementary photo 8) \(10) Simulation of in vitro renal preservation \(CS group) Before heading to 1h, release the tissue forceps 30min in advance, open the abdominal cavity to expose the left kidney, and be cautious to protect the intestinal tract. At this time, Change 5% glucose solution to hydroxyethyl starch solution I.V. 3 s/drop. Carry out abdominal transverse incision to make cutting edge in parallel with the left renal pedicle, the length is 7-9cm, the transverse incision and the original incision should be shaped like "T", and then stop the bleeding. After completion of hemostasis, give intravenous bolus injection of 0.3ml heparin \(1875 IU) via the right marginal ear vein, and then prepare the renal artery puncture device \(venous indwelling needle, infusion set and 4°C heparinized citric acid renal protection solution). Isolate the left kidney with the surrounding tissues. Do not cut the ureter and renal pedicle. Perform the ligation to hemostasis, and prevent the infiltration of blood in surrounding tissue. At the moment heading to 1h, clip the renal artery and vein by artery clamp at the same time, and then clip the ureter by the other artery clamp. Then tie two 3-0 mousse line in the renal artery for backup. The left hand pulls the line proximal to the heart, and the right hand punctures the renal artery with head of intravenous indwelling needle. Prepare the crushed ice, 4°C renal preservation solution in citric acid and renal bags. After completion of puncture, open the infusion set, and then prick gently the renal vein with the head of venous indwelling needle after the kidney becomes white, at this time, water column will emit. Observe the perfusing speed preventing the indwelling needle slippage or obstruction. Start countdown for 4h. Firstly ligate the distal end neither too tight nor too loose, to prevent slippage, and then ligate the proximal end. Place the kidney into the bag filled with 4°C citric acid renal preservation solution, tighten the renal bag, and then make sure the bag should not be too tight to prevent the indwelling needle obstruction. Observe the perfusion velocity. Take out the kidney bag from the left body of rabbit, and then perform in vivo renal preservation in the round plate filled with mixture of ice and water. Suture “T” shaped cut, and the kidney was persevered in vivo for 4 hrs. \(Supplementary photo 9, 10) \(Supplementary video 1) \(11) After completion the 10th step -simulation of in vitro renal preservation \(MP group), begin to prepare the

drainage tube and lifeport perfusion apparatus. Move the rabbit in proper position so that the left kidney can be pulled out from the transverse incision, but the renal vessels and ureter should not be pulled off. Make sure the streaming velocity does not change, and then place the drainage tube inside, afterwards suture the transverse skin incision to close the left abdomen, which should not influence the streaming velocity. Place the kidney into the sterile plate filled with crushed ice, and bury it with crushed ice. Keep the kidney hypothermia all the time. Install lifeport perfusion apparatus for hypothermia machine perfusion preservation. Afterwards, suture the skin of abdominal median incision, and restore the abdominal temperature. Constantly observe the perfusion flow and the running status of lifeport perfusion apparatus \(pressure is 60mmHg, and flow rate should be controlled at 15-20ml/min). At this moment, the hydroxyethyl starch solution can be changed to 5% glucose solution \(5s/drop). Monitor the vital signs in the rabbit for 4hrs; make sure there is plenty of ice in the plate. To determine the drainage tube is unobstructed, perform the second blood gas analysis when preserving 2hrs. \(Supplementary photo 11) \(Supplementary video 2) \(12) Vascular Sutures Carry out the third blood gas analysis 20 min in advance before heading to 4h, meanwhile prepare 50ml 4°C Lactated Ringer's solution, 5ml sodium bicarbonate, 2ml heparin, 2ml furosemide, 50ml heparin sodium chloride and 50ml 4°Cnormal saline. Open midline incision and the left abdominal transverse incision 5 min in advance \(for MP group, lifeport perfusion apparatus needs to be removed to stop pouring). At the same time, change to hydroxyethyl starch solution I.V. 1 s/drop. Fill 50ml 4°C Lactated Ringer's solution in the kidney to rinse away the residual citric acid renal preservation solution. First suture the vein via 10-0 sized suture needle with prolene; then suture the artery after removing the indwelling needle with 10-0 sized suture needle with prolene. The moisture and temperature should be kept in the intestine during the vascular suture, and pay attention to the vital signs of the rabbit. After completion of the vascular suture, carry out leakage test of clipping distal end of renal artery and vein, the procedure is as the followings: open two artery clamps, and determine whether there is bleeding and stenosis in artery and vein. Recover the renal blood supply. At this moment, give intravenous bolus injection of 0.2ml heparin and 0.5ml furosemide via the right marginal ear vein, and 5ml 5% sodium bicarbonate slowly. Observe the vital signs and kidney status of rabbit. Conduct the fourth blood gas analysis. Close the left abdominal transverse incision carefully layer by layer. Prepare the fistula right now. \(Supplementary photo 12, 13) \(13) Bladder Stoma. Determine the fistula position, use the purse-string suture, and make sure the bladder should not be strained to injury. At this moment, give intravenous bolus injection of 0.5ml furosemide through the marginal ear vein. \(14) Right Kidney Resection Separate the right renal pedicle and make double ligation; separate the ureter and make double ligation; release the tissues around the kidney, and make ligation for hemostasis. Cut off the renal pedicle and ureter. Resect the right kidney and stop bleeding. At this moment, give intravenous bolus injection of 0.5ml furosemide through the marginal ear vein. \(Supplementary photo 14) \(15) Sort and count the apparatus, rinse the abdominal cavity, and close it carefully layer by layer. Then replace hydroxyethyl starch with lactated Ringer's solution I.V. 5 s/drop. Loosen the rabbit from the fastening device, and dry it with blower to make it restore the body temperature as soon as possible. Clean up the experimental apparatus & equipment, clean the perfusion laboratory, and disinfect with UV for backup. **Postoperative maintenance** After the operation, monitor the vital signs, urine volume and blood gas analysis of rabbit for 24hrs. Correct the disorder of acid-base

balance timely, and supplement the blood volume, which will contribute to the recovery of left kidney function. **Collecting specimens** Measure the urine volume 24h after operation. Collect the rabbit's blood into a tube with anticoagulant, and centrifuge 5 min at 2000 rpm to collect the plasma. Content of blood urea nitrogen \(BUN) and creatinine \(Cr) should be tested with automatic biochemical analyzer; collect another blood into the tube without anticoagulant, and centrifuge 5 min at 2000 rpm to collect the serum, and test the content of TNF-o, IL-6 with ELISA kit; collect tissue to detect the cell apoptosis by TUNEL. \(Supplementary photo 15) **Statistical analysis** Analyze the data using SPSS17.0 statistical software, and the data are normally distributed through normality test. Measurement data are expressed by mean± standard deviation \(X±S). Analyze the inter-group data using one-way ANOVA, and apply analysis of variance in a repeated measures design on the intra-group data. α=0.05, $p < 0.05$ is considered that the difference is statistically significant.

Timing

200 min

Anticipated Results

1. 24- hour survival rate The rabbits revived 1 h after finishing the experiment, and their24-hour survival rates were observed thereafter. There were no statistical differences in 24-hour survival rates between MP25min and CS25min group \(Figure 1A), MP45min and CS45min group \(Figure 1C) \($p>0.05$). The results of both 25min groups were 100%, while both the 45min groups were 0%. However, the 24-hour survive rates between MP35min and CS35min group were significantly different \($p<0.05$), while the former was 100%, the latter was 80%. \(Figure 1B) **2. Early graft function after reperfusion for 24 hours.** Collect the rabbit urine of all the groups at corresponding time points, and measure the volume. The results showed, 24 hour urine volume of normal rabbit was 50 ± 5 ml, that of CS25min group was 50 ± 10 ml, while that of MP25min group was 150 ± 10ml, the difference was statistically significant \($P < 0.05$). 24 hour urine volume was 35 ± 8 ml for CS35min group, 110 ± 20 ml urine for MP35min group, and there was statistical difference \($P < 0.05$); 24 hour urine volume was 8 ± 2 ml for CS45min group, 10 ± 20 ml urine for MP45min group, and there was no statistical difference \(Figure 2A) \($P > 0.05$); After the operation, take the blood from all the groups, and test the blood creatinine with biochemical analyzer. The results showed, creatinine of CS25min group was 480 ± 23 μmol/L, that of MP25min group was 300 ± 14 μmol/L, the difference was statistically significant between the two groups \($P < 0.05$); creatinine of CS35min group was 511 ±44 μmol/L, while that of MP35min group was 355 ± 71 μmol/L, the difference was statistically significant between the two groups \($P < 0.05$);The rabbits of MP45minand CS45min group did not survive to 24 hours, so the blood samples were drawn from nearly dead rabbits. The test results revealed, creatinine of CS45min group was 370 ± 43 μmol/L, that of MP45min group was 330 ± 36 μmol/L, the difference was not statistically significant \(Figure 2A) \($P > 0.05$). **3. Diameters of renal tubules and thickness of renal tubular epithelial cells** By HE staining, thickness of renal proximal convoluted tubular epithelial cells \(9.655±1.877μm) and distal convoluted

tubular epithelial cells \(6.154±1.23μm) in the MP 25min group decreased when compared with the CS 25min group \(12.566±1.877μm and 8.755±1.43μm, respectively) \(Figure 3A and 3B) \(P<0.05). Thickness of renal proximal convoluted tubular epithelial cells \(12.338±1.833μm) and distal convoluted tubular epithelial cells \(10.870±1.686μm) in the MP 35min group decreased when compared with the CS 35min group \(18.377±2.108μm and 14.075±2.662μm, respectively) \(Figure 3C and 3D) \(P<0.05). Thickness of renal proximal convoluted tubular epithelial cells \(24.344±3.120μm) in the MP 45min group decreased when compared with the CS 45min group \(26.899±3.650μm) \(Figure 3E and 3F) \(P<0.05), while thickness of distal convoluted tubular epithelial cells showed no significantly difference between MP 45min group \(22.500±3.322μm) and CS 45min group \(23.320±3.240μm) \(Figure 3E and 3F) \(P>0.05). **Conclusion** At present, mechanism of hypothermic machine perfusion has not been fully understood, therefore many researchers have made a lot of effort to have some animal models established, mostly pigs, dogs, and other large animals were used in the study, such as: AnjaGallinat, Nader Vaziri et al \[15, 24] have made allogenic porcine kidney transplantation, and Susanne Lindell et al \[25] have used dog to study the protection of UW solution for donor kidney. Gregory M. Fahy and Suja E. Ali have made normothermic perfusion of rabbit kidney \[26], however it is not reported that rabbit was used as model to study the mechanism of hypothermia machine reperfusion. Prior to establishing the model, Hauet T et al \[27] introduced the pig kidney auto-transplantation model making method, which was different with in vivo machine perfusion model. It eliminated the immunological rejection caused by allogeneic renal allografts, therefore autologous model can exclude the interference of immune system. In the model we established, the kidney was not completely isolated from the body, which makes the blood vessel suture simplified and the suture time shortened \(average 5min), and reduces the vascular damage. This study is a bold attempt; fortunately, the complete animal model making method is stable and reliable after repeated exploration of the author. In order to ensure the survival of rabbits, the rules and procedures of experimental operation should be followed strictly; besides that, there are still some cautions: 1) the weight of rabbit has a significant effect on the survival rate. The study revealed that rabbit of less than 3.0kg body weight after fasting has poorer tolerance; after the operation, it’s difficult to maintain the life sign and very easy to die. Therefore, selecting the rabbit of more than 3.0kg body weight \(better more than 3.2 kg) is one important factor to guarantee postoperative survival; 2) strict preoperative fasting and clean environment in laboratory are mandatory for successful experiment. It’s very likely for them to have mesenteric congestion and intestinal swelling during the operations, which are difficult to recover after the operation; also intestinal adhesion obstruction is liable to occur which can cause disorder of water & electrolyte and acidosis, so the survival rate decreases \(CAUTION: the intestinal tissue and mucous membrane exposed in the operation should be continuously covered with the gauze soaked in warm saline, otherwise it would have the same consequences); 3) Be sure to clip the ureter, which can prevent input and return of renal blood through ureter artery and vein, otherwise the renal warm ischemia is not sufficient, and hypothermic machine perfusion is not finished completely, so that it will lead to reduced reliability of the experiment. During the operation, heparin was used many times. It was used for the first time to make donor kidney heparinized to prevent residual blood coagulation in the kidney after clipping the renal artery and vein, which can cause embolism after opening again; it was used for the second time to reduce the incidence of donor renal embolism; 4) The

renal injury is diffident with varying warm ischemia time in rabbits. For the machine perfusion model group of donor renal warm ischemia 25 min, 24 hour survival rate was 100%, and the urine volume was nearly normal, but the longer warm ischemia time is, the severer kidney damage will be; the less urine volume it is, and survival time will also be shortened, and survival rate decreased. The results show that the model has very good stability.

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Figures

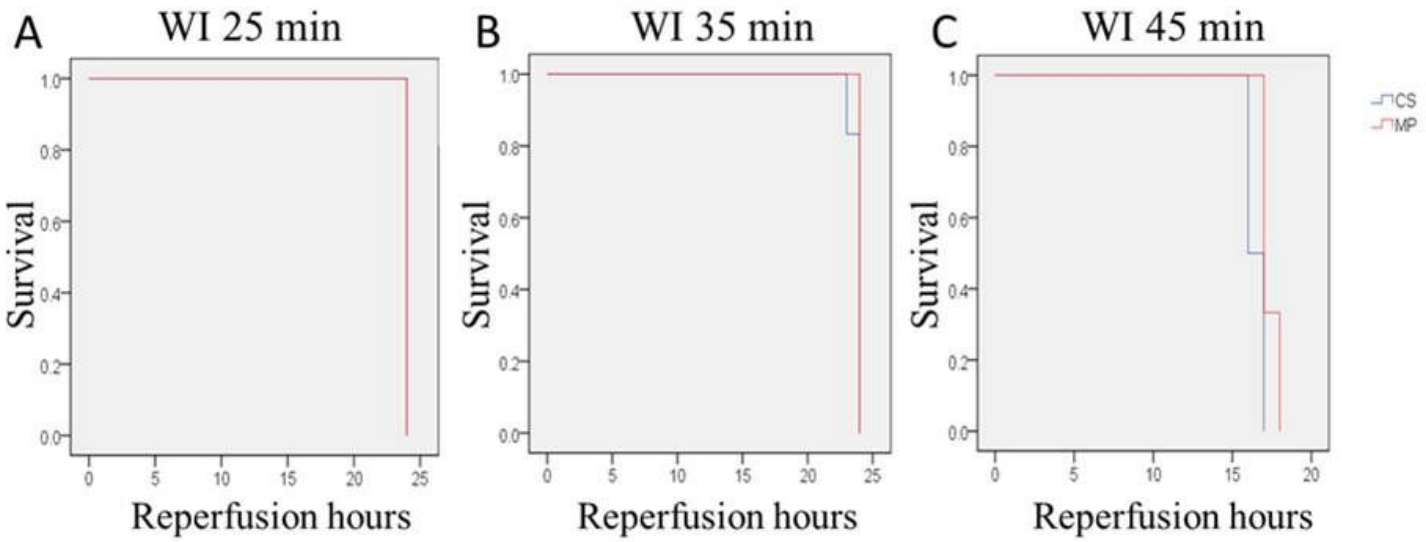

Figure 1

24- hour survival rate 24- hour survival rate

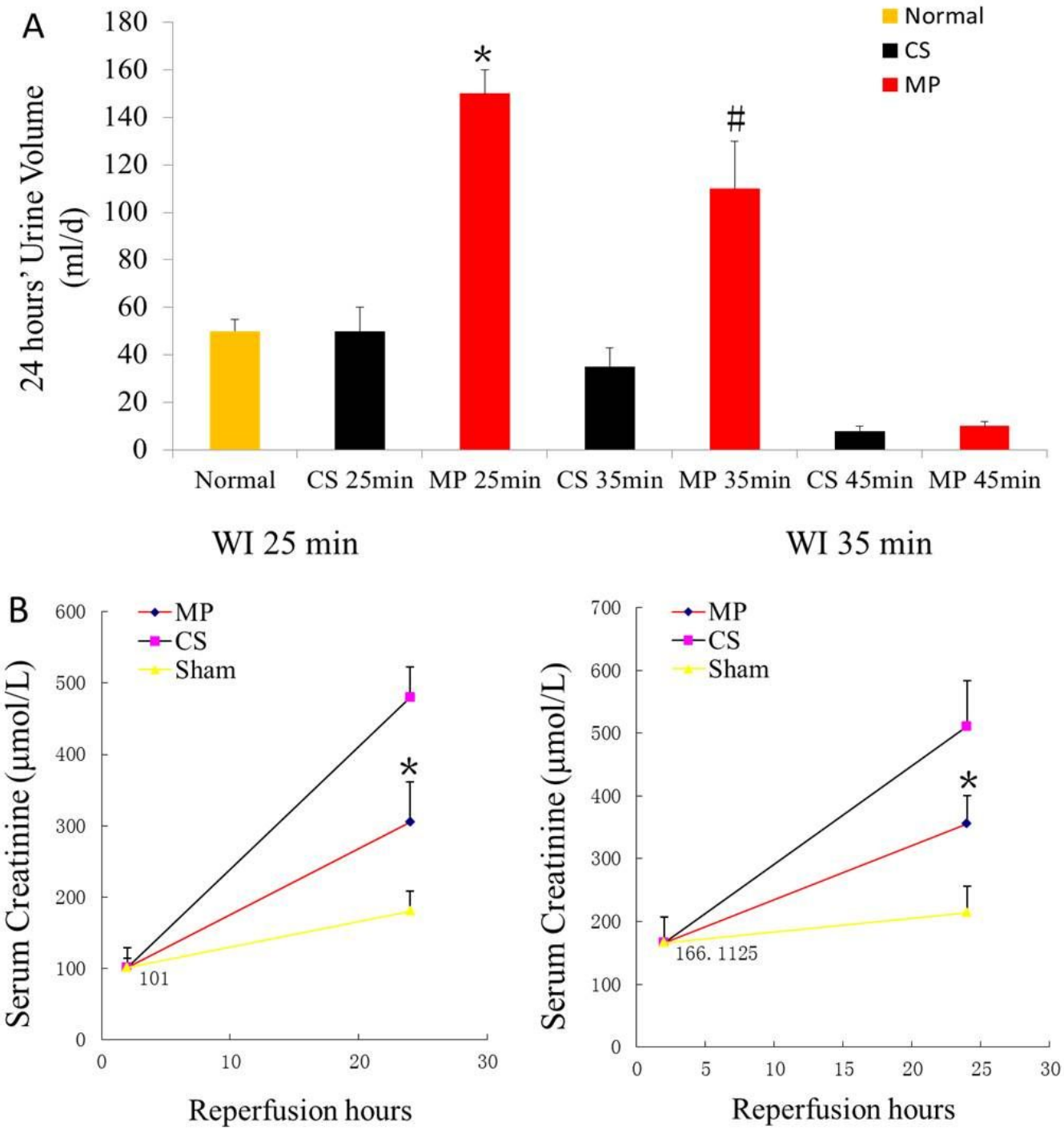

Figure 2

Early graft function after reperfusion for 24 hours. Early graft function after reperfusion for 24 hours.

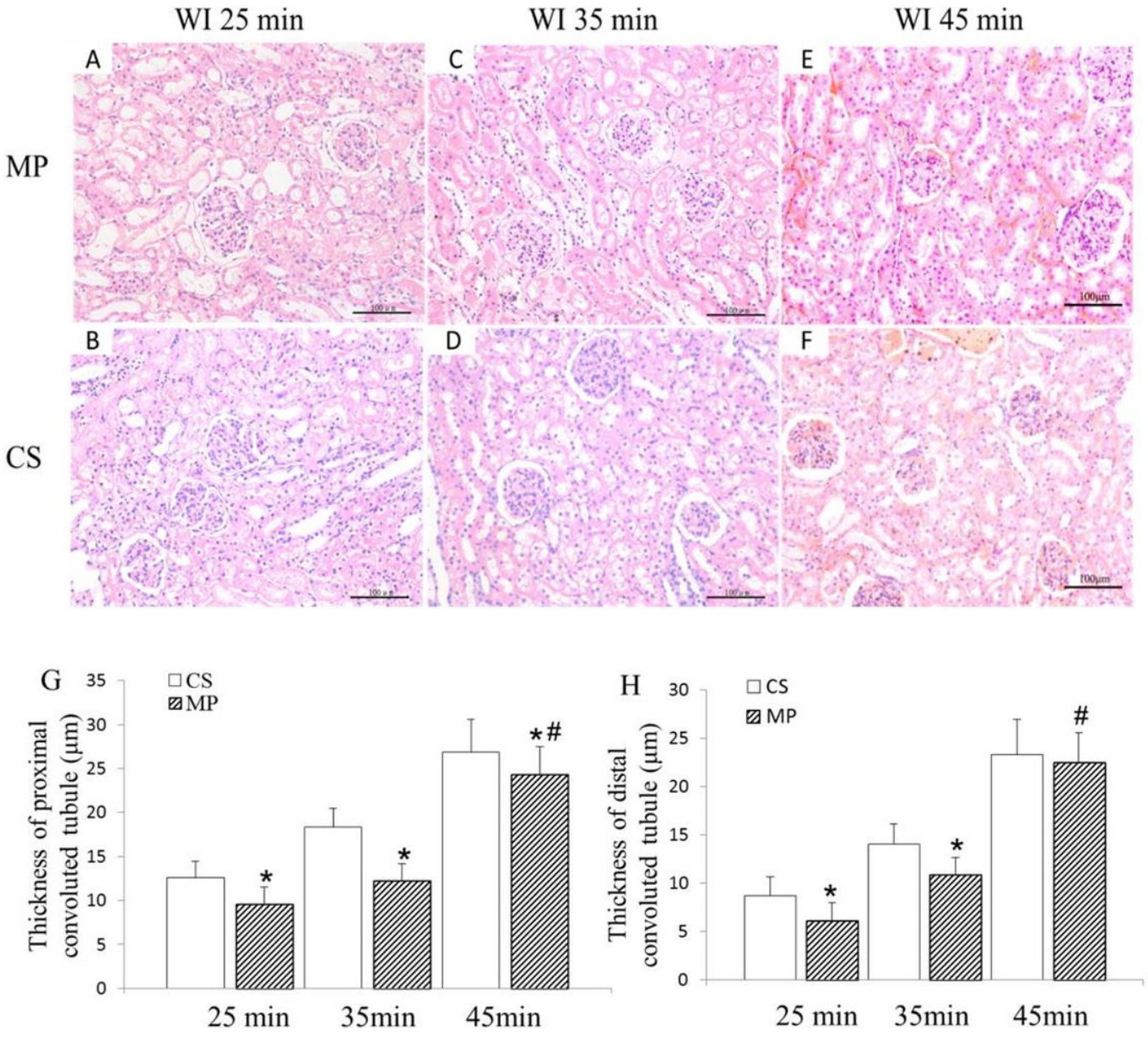

Figure 3

Diameters of renal tubules and thickness of renal tubular epithelial cells Diameters of renal tubules and thickness of renal tubular epithelial cells

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