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ORIGINAL ARTICLE

Magnetic ring anastomosis of suprahepatic vena cava: novel technique for liver transplantation in rat

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Summary

To improve the technique of suprahepatic vena cava (SHVC) reconstruction in rat OLT, novel magnetic rings were designed and manufactured to facilitate reconstruction of SHVC and shorten the anhepatic time. One-hundred and twenty adult male Wistar rats were randomly divided into two groups: rings group ($n = 30$), using magnetic rings for SHVC reconstruction; suture group ($n = 30$), 7/0 prolene suture was used for SHVC running anastomosis as control. Cuff techniques were used for portal vein and infrahepatic vena cava reconstruction as Kamada and Calne described. The bile duct was reconnected with a stent. The hepatic re-arterialization was omitted. In the rings group, the SHVC reconstruction took 0.91 ± 0.24 (mean $\pm$ SD) min; the anhepatic phase and the recipient operation time were 5.63 ± 0.65 min and 36.02 ± 8.02 min, respectively. In suture group, the anastomotic time of SHVC was 10.40 ± 2.11 min; the anhepatic phase and the recipient operation time were 17.76 ± 2.51 and 49.38 ± 12.06 min, respectively, and there was statistically significant difference between the two groups. The ALT levels reached peak at 24 h post-OLT (186.2 ± 32.5 IU/l) and restored to normal level at 96 h gradually. In the rings group, 29 of 30 rats survived at day 7 and 28 of 30 rats survived at day 30. In contrast, only 25 of 30 recipients in suture group remained alive at day 7 and 22 of 30 remained alive at day 30 ($P < 0.05$). Better anastomotic healing was founded in rings group by pathology and scanning electron microscope. The magnetic rings technique provides a novel, simple method for SHVC reconstruction of OLT in rat. It significantly shortens anhepatic phase, while the success rate of the operation is satisfactory.

Introduction

Nowadays, orthotopic liver transplantation (OLT) in rat has been generally accepted as an excellent model for research in the field of organ preservation, transplant pathology, physiology, and immunology. It was first described by Lee *et al.* in 1973, using hand-suture techniques [1]; however, it was not widely used due to the prolonged anhepatic phase and technical demand. In 1979, Kamada and Calne [2] reported “two-cuff technique” for the portal vein and infrahepatic vena cava, and their technique shortened anhepatic phase to 15–20 min and increased 1-week survival rates to 70% successfully, which became globally accepted [3]. However, the suprahepatic vena cava (SHVC) anastomosis remains difficult to be performed, and skillful microsurgical suturing techniques and long learning curve are required. In 1980, Miyata introduced the “three-cuff model” for the three venous anastomoses [4]. However, in this method, the diameter of the intrathoracic segment of the vena cava was too thin to adequately handle the necessary flow; furthermore, there was a risk of kinking and torsion. It has not gained wide acceptance. Tsuchimoto et al. [5] and Harihara Y [6] also described cuff method for SHVC anastomoses. However, it is fairly difficult to insert the cuffed donors' SHVC into the recipients' SHVC, and the ligation is difficult to be performed due to the short anterior wall of the recipients' SHVC. These shortcomings also exist in other studies using cuff technique for SHVC anastomosis [7,8]. OLT in rat is the most popular model for liver transplant research. Minimizing the anhepatic time is the key of a successful OLT in rat.

Magnetic compression techniques have been described for various indications such as hepatobiliary, gastroenteral, or vascular anastomoses. The technique has been described in animals and a few clinical studies. Its concept still seems to be an intriguing idea, but a worldwide breakthrough does not happen so far. Based on our research of previous magnetic compression techniques [9], we have developed the novel magnetic rings for SHVC construction in rat OLT model to avoid the disadvantages of suture techniques or cuff method.

Materials and methods

Device design

Magnetic rings made from neodymium–iron–boron, which has been widely used in compression anastomosis [10–12]. The rings are designed by ourselves as Fig. 1 shows, weighing 0.15 g, and manufactured in factory. The rings are curved to the desired shape by wire-electrode cutting technique, specially coated by a titanium oxide airbrush and sterilized by 10% povidone–iodine solution.

Animals and groups

One-hundred and twenty adult male Wistar rats, weighting 230–280 g, were selected as donors and recipients, which were randomly divided into two groups: rings group ($n = 30$), using magnetic rings for SHVC reconstruction; suture group ($n = 30$), using hand-suture technique for SHVC anastomosis as control.

Surgical techniques

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Tianjin First Center Hospital (Permit Number: A2012-362). All animals were anesthetized with sevoflurane anesthesia, and all efforts were made to minimize suffering.

Donor operation

The donor operation, including the recovery and preparation of the donor liver, was performed according to Kamada and Calne [2]. The abdomen was opened via midline incision, and Teflon catheter was inserted into bile duct as stent and fixed with 5-0 silk suture. The liver was flushed with 4 °C Ringer's solution, and the donors' SHVC including diaphragm ring was dissected.

Back-table graft preparation

After donor liver recovery, the magnetic rings in rings group was prepared as follows: the ends of the SHVC were everted over the rings and secured in proper position of the rings by diaphragm loop, as shown in Fig. 2a–c. The lumen was flushed with saline. Cuffs were prepared for portal vein and infrahepatic vena cava as Kamada and Calne described [2]. The hepatic artery was ligated.

Recipient operation

The recipient's abdomen was opened via a midline incision. The liver was mobilized from the ligaments around it. The right suprarenal vein and lumbar vein were ligated and divided. The hepatic artery was doubly ligated and divided. The bile duct was dissected free from the portal vein. After the portal vein and infrahepatic vena cava were cross-clamped, the SHVC was cross-clamped with a Satinsky clamp, exceeding the diaphragm ring. The recipient's SHVC was cut under the confluence level of the hepatic veins. After the recipient's liver was removed, the donor liver was placed in situ and covered with cold wet swab.

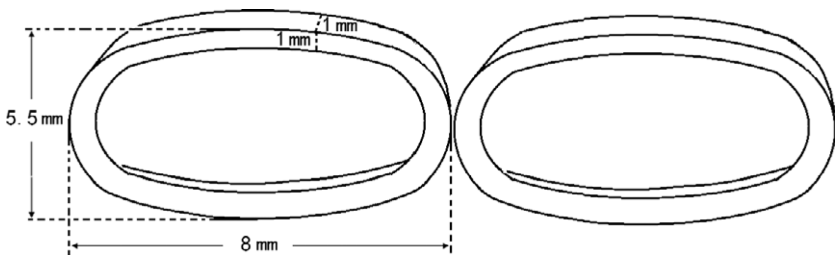

Figure 1 Coupled magnetic rings for suprahepatic vena cava reconstruction.

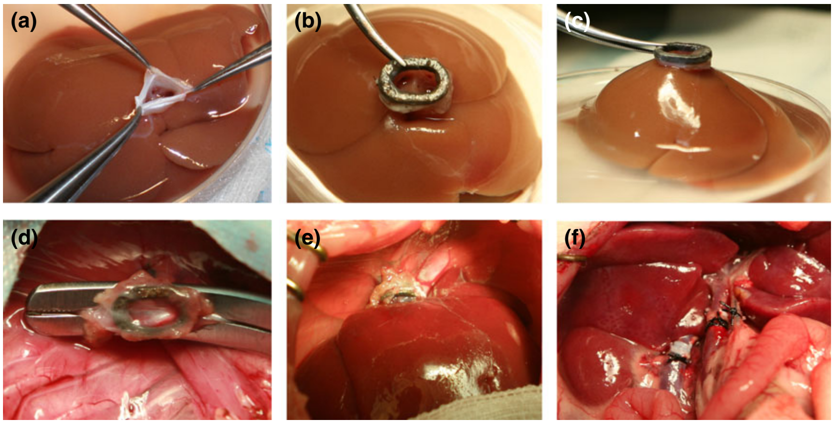

Figure 2 Liver transplantation procedures using magnetic rings. (a) Preparation of donor's SHVC. (b) (c) End of the donor's SHVC were everted over the ring's and secured in the proper position of the rings by diaphragm loop. (d) End of recipient's SHVC was everted through the magnetic ring and secured in the proper position, and the ring was fixed on Satinsky clamp by magnetic attraction. (e) Anastomosis site of SHVC after reperfusion in group a. (f) Liver and kidney after reperfusion in group A.

In rings group, end of recipient's SHVC was everted through the magnetic ring and secured in proper position, and the ring was fixed on Satinsky clamp by magnetic attraction, as shown in Fig. 2d. The lumen of the donor's SHVC was cleared off the blood and filled with Ringer's solution to remove air bubbles and prevent air embolism. Then, the SHVC reconstruction was completed after the coupled rings embedded into the ends of the donor's and recipient's SHVC through being attracted together automatically by magnetic power. In suture group, the donor's SHVC was anastomosized end-to-end to the recipient's SHVC by continuous 7-0 prolene suture. Cuff techniques were used for portal vein reconstruction in rings group and suture group as Kamada and Calne described [2]. After washing out with saline solution, the cuff of donor's portal vein was inserted into the lumen of recipient's portal vein. The anastomosis was completed with a circumferential 6-0 silk suture.

As the clamps on portal vein and SHVC were released, the graft liver regained blood supply, and the anheptic phase was over, as shown in Fig. 2e. Then, the same operation procedures were performed for infrahepatic vena cava reconstruction, as shown in Fig. 2f. The bile duct was connected end-to-end via Teflon catheter and fixed with 5-0 silk suture, and the anastomotic site was wrapped with omentum tissue to prevent bile leak. No attempt was made to reestablish the blood flow of the hepatic arterial. Finally, the abdominal incision was closed with a continuous 3-0 suture.

Postoperatively, the animals got rewarming with incandescent lamp in their cages for 1 h, and 100 mg penicillin per kg was injected i.m. No immunosuppressive agent was given.

Table 1.

	Group A (n = 30) Magnetic rings	Group B (n = 30) Suture	P value
SHVC anastomosis (min)	0.91 ± 0.24	10.40 ± 2.11	0.000
Anheptic time (min)	5.63 ± 0.65	17.76 ± 2.51	0.002
Recipient operation (min)	36.02 ± 8.02	49.38 ± 12.06	0.032

Statistical analysis

All data are expressed as mean ± standard deviation (SD). Animal survival was evaluated by Kaplan–Meier method and log-rank test. Data were analyzed with Student's t-test for unpaired data. The P value of <0.05 was considered statistically significant.

Results

In rings group, the SHVC reconstruction using magnetic rings took 0.91 ± 0.24 min, while the anhepatic phase and recipient operation time were 5.63 ± 0.65 min and 36.02 ± 8.02 min, respectively. In suture group, the SHVC anastomosis time was 10.40 ± 2.11 min, while the anhepatic phase and recipient operation time were 17.76 ± 2.51 and 49.38 ± 12.06 min, respectively. Significant difference had been showed between these two groups as showed in (Table 1).

We had observed no thrombosis or bleeding at the SHVC anastomotic site in rings group. Color Doppler ultrasound had showed the blood flow of the hepatic vein was normal (as shown in Fig. 3), and the inferior vena cavography was also normal (as shown in Fig. 4). The ALT levels reached peak at 24 h post-OLT (186.2 ± 32.5 IU/l)

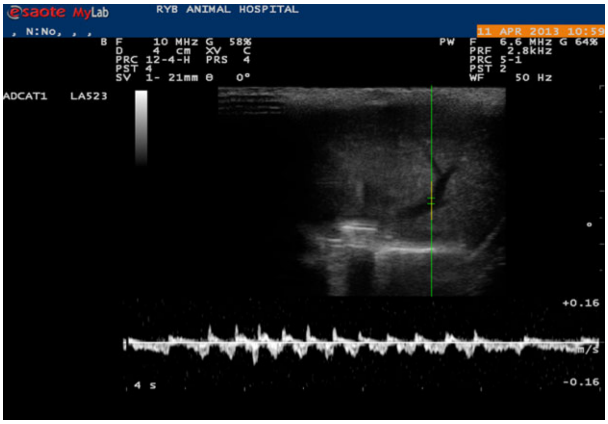

Figure 3 B-ultrasound observation of hepatic veins in the rings group, 2W postoperation.

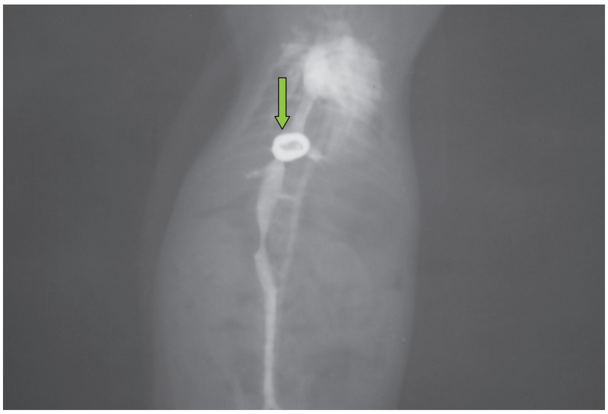

Figure 4 Inferior vena cavography in the rings group, 2W postoperation.

and restored to normal level at 96 h gradually. Better anastomotic healing was founded in rings group by pathology and scanning electron microscope (as shown in Fig. 5).

In the rings group, 29 of 30 rats survived at day 7, and 28 of 30 rats survived at day 30. In contrast, only 25 of 30 recipients in suture group remained alive at day 7, and 22 of 30 remained alive at day 30 ($P < 0.05$) (as shown in Fig. 6).

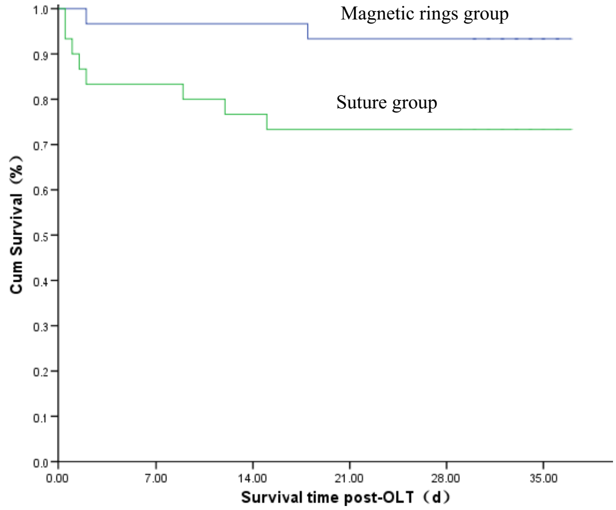

Figure 6 Novel magnetic rings for SHVC construction prolong rat orthotopic liver transplant (OLT) survival ($P = 0.034$).

Discussion

The needle and thread are still used for vascular anastomosis, more or less as described 100 years ago. Suturing, however, has several detrimental aspects. The penetrating needle induces vascular wall damage, which influences the healing response. Nonabsorbable suture material is left as an intraluminal foreign body and may cause inflammatory reaction, thrombocyte aggregation, impaired endothelial function, intimal hyperplasia, and hence stenosis [13]. Moreover, performing sutured vascular anastomoses demands extensive training if high patency rates are to be achieved [14], especially to OLT in rat, regarding as a relatively difficult model.

Though two-cuff techniques for portal vein and infrahepatic IVC described by Kamada and Calne [2] had improved long-term survival rates of higher than 90%. SHVC anastomosis for OLT was still difficult, and the obvious difference of anhepatic phase would impact on our results. Consequently, search for new methods resulting in faster and easier vascular anastomoses was intriguing to transplant researchers.

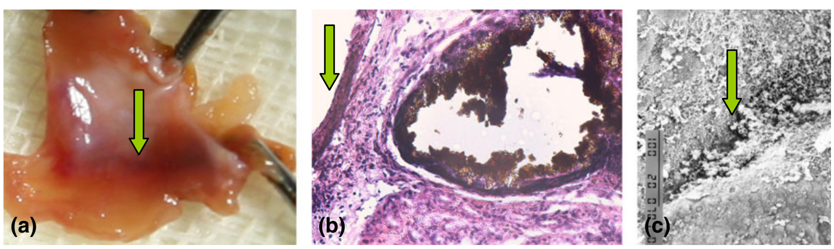

Figure 5 Anastomotic site of SHVC in magnetic rings group. (a) Naked eye 2W postoperation, (b) HE stained image 2W postoperation, and (c) Scanning electron microscope 2W postoperation.

Magnetic compression techniques have shown promise in biliary and vascular anastomoses (although the latter involves permanent implantation) [15,16]. The technique has been described in animals and a few small clinical studies. Its concept still seems to be an intriguing idea, but a worldwide breakthrough does not happen so far. Based on our previous research of magnetic compression techniques [9], we developed novel magnetic rings for SHVC construction in rats to avoid the disadvantages of suture techniques or cuff method.

Using our technique, SHVC construction in rats OLT model became simple and fast, SHVC reconstruction time decreased from 10.40 ± 2.11 min to 0.91 ± 0.24 min, anhepatic phase decreased from 17.76 ± 2.51 min to 5.63 ± 0.65 min. We observed no thrombosis or bleeding at the anastomotic site in rings group, and good healing had been proved by scanning electron microscope. Moreover, we achieved prolonged survival rate than suture technique.

In the cuff method for SHVC anastomoses [4–8], the SHVC cuff was round shape, the inner diameter was too thin (1.8–3.5 mm) to supply adequate blood flow, the height was too long to avoid the risks of kinking and torsion, it is fairly difficult to insert the cuffed donors' SHVC into the recipients' SHVC, and the ligation is difficult to be performed due to the short anterior wall of the recipients' SHVC. In our technique, the magnetic rings for SHVC reconstruction was like oval shape, long inner diameters was 6 mm and short diameters was 3.5 mm, could supply adequate blood flow, fitting the anatomy characteristic of SHVC. The height was only 2 mm, could reduce the risks of kinking and torsion obviously. SHVC reconstruction was performed by powerful magnetic attraction of rings, avoiding the difficulties of insertion and ligation, avoiding the risk of separation.

This technique could not be used when the SHVC is nearly inexistent, such as Fischer F344 or Buffalo. However, it has been used for Lewis (donor) to brown Norway (recipient) rats successful. Pitfalls of this technique include the following: need patient to secure the ring to the graft's SHVC carefully, avoid twisting when everting the recipient's SHVC, remove the bubble of SHVC thoroughly, etc.

In conclusion, the magnetic rings technique provides a novel, simple method for SHVC reconstruction of OLT in rat. It significantly shortens anhepatic phase, while the success rate of the operation is satisfactory.

Authorship

YS, WZ, YD, YZ, QZ, WZ, HZ, CP, ZS: participated in research design. YS, WZ: participated in the writing of the paper. YS, WZ, YD, ZS: participated in the performance of the research. YS, WZ: contributed new reagents or analytic tools. YS, WZ: participated in data analysis.

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The authors have declared that no competing interests exist.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Movie S1 Back-table graft preparation in rings group.

Movie S2 Recipient operation in rings group.

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