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An experimental study on establishment of a high-volume common carotid-external carotid bypass model in dogs

Lin Ma, Hecheng Ren, Jialin Li, Xiangmao Zhang and Ying Huang*

Department of Neurosurgery, Tianjin Huanhu Hospital, Tianjin, China.

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High-volume intra- and extra cranial artery bypass surgery has been widely used, but it remains a difficult technique for most of surgeons. The objective of this study is to explore a training mode and platform. Six healthy dogs were divided into three groups at random. Twelve high-volume common carotid-external carotid bypasses were operated on both sides of six dogs (n=12). Digital subtraction angiography (DSA) and color Doppler sonography were taken to measure the hemodynamic parameters of grafts one week (n=4), four weeks (n=4) and 24 weeks (n=4) after the operations. The grafts were taken out for histopathological examination 24 weeks after the operations. The high-volume common carotid-external carotid bypass model (n=12) was successfully established in six dogs under microscope. Post-operative DSA in Week 1 (n=4), Week 4 (n=4) and Week 24 (n=4) showed bilateral external carotid blood flow was supplied by contralateral common carotid grafts. Both ends and the whole grafts were patent. Color Doppler sonography showed clearly that the blood flow volume of grafts was higher than 90 ml/min. Histopathological examination taken 24 weeks after the operations showed intimal hyperplasia of grafts. Dog model of high-volume common carotid-external carotid bypass has the advantages of similar material, high volume, similar blood pressure, feasible operation and high patent rate compared to the traditional microsurgery model, making it a better simulation training platform which is closer to the real surgical procedure for surgeons willing to master the technique of high-volume bypass operation.

Key words: Dog, animal model, bypass, high-volume, vascular anastomosis.

INTRODUCTION

In recent years, extra cranial–intracranial bypass has been playing a more and more important role in the treating of diseases including ischemic cerebrovascular diseases, complicated aneurysms and skull base tumors (Eliason et al., 2002; Evans et al., 2004; Deshmukh et al., 2005; Mohit et al., 2007; Sekhar et al., 2008; Patel et al., 2010; Xu et al., 2011; Ramanathan et al., 2012; Kalani et al., 2013; Lougheed et al., 1971). The indications for this technique are usually classified into two categories, flow replacement and flow augmentation (Amin-Hanjani, 2011).

*Corresponding author. E-mail: malinsci@126.com

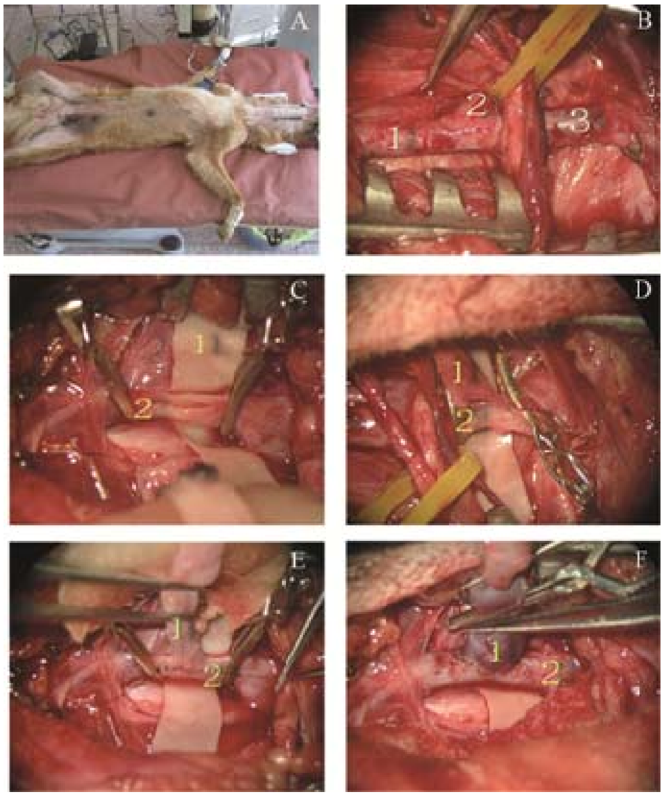

Figure 1. (A) Exposure of incision: cervical incision, lower-limb incisions and body posture. (B) Exposure of cervical vessels: 1 common carotid; 2 internal carotid; 3 external carotid. (C) The anastomosed position on the common carotid and the incision(arrow) on the artery. (D) Anastomosis(arrow) of the posterior wall was completed and the clamps were not released yet. (1) The common carotid. (2) The graft. (E) Anastomosis (arrow) of the graft was completed and the clamps were not released yet. (1) The common carotid. (2) The graft. (F) The anastomosis (arrow) was completed and the clamps were released with no leakage. (1) The common carotid. (2) The graft.

While technically, there are two modalities of extra cranial–intracranial bypasses (Amin-Hanjani et al., 2010). The first is termed as “high-flow” bypass usually using a vessel graft. The commonly used grafts include the saphenous vein and radial or ulnar arteries.Bypass usingradial artery is sometimes referred as “intermediate-flow” (Prinz et al., 2014). The second is termed as “low-flow” bypass usually using in situpedicles. The superficial temporal artery to middle cerebral artery revasculation is a typical “low-flow” bypass.

Nowadays, “high-flow” bypass is becoming the research focus of neurosurgeons. For example, endovascular stenting has been used for the management of graft stenosis (Maselli et al., 2011), and self-closing U-clips used for interrupted intracranial microanastomosis (Ferroli et al., 2007). The objective of this study is to establish a dog model of high-volume common carotid-external carotid bypass for peri-operative observation, to explore a training mode for the popularization of high-volume intra- and extra cranial artery bypass and to provide a simulation training platform for concerning neurosurgeons, vascular surgeons and skull base surgeons.

MATERIALS AND METHODS

Experimental animals

Six healthy dogs, male or female, with weight of 10 to 12 kg were provided by the animal experiment center of Tianjin People’s Hospital.

Randomization

Six dogs were sequentially numbered (No.1 to 6) and allocated into three groups at random. DSA and color Doppler sonogram were planned 1 week (for No. 1 and 4 only), 4 weeks (for No.2 and 5 only) and 24 weeks (for No. 3 and No. 6 only) after the operation. The vascular anastomosis was completed with interrupted suture for No.1 and No.2 dogs or continuous suture for No. 3 to 6 dogs.

Pre-operative preparations

Aspirin 300 qd was administered for three days before the operation. Preoperative fasting for 12 h was mandatory but the dogs had free access to water.

Skin preparation

Skin preparation was completed under basal anesthesia 30 mins before the surgery. The skin area included cervix and bilateral femoral regions. The cervix area covered the middle part of mandibular body anteriorly manubrium posteriorly and body surface projection of the transverse process of cervical vertebras laterally. Bilateral femoral regions were bounded superiorly by inguinal ligament and inferiorly by knees and laterally by lines 5 cm away from the incision (Figure 1A).

Anesthesia

Intramuscular atropine 0.05 mg/kg and ketamine hydrochloride 10 mg/kg were injected for basal anesthesia. After anesthesia induction with intravascular hydrochloride 2 mg/kg and propofol 1 to1.5 mg/kg, oral intubation were performed using laryngoscope (inner diameter: 6.5 to 7.5 mm) with muscle relaxation. Propofol and enflurane were used for anesthesia maintenance.

Model establishment

Cervical incision

At supine position on the operating table, the neck of the dog was exposed adequately with fixation of limbs, hyperextension of the head and pillow under the cervix (Figure 1A). After sterilization with iodine and alcohol and draping, the incision was made. Along the anterior boundary of musculus sternocephalicus, straight incisions were made to cut open the skin and subcutaneous tissue bilaterally.

The connective tissue surrounding musculus sternocephalicus, sternothyroid and the sternohyoideus were bluntly dissected to expose the common carotids on both sides. Then the common carotids were dissected distally to the bifurcation to expose 1 to 2 cm of external carotids (Figure 1B) for future anastomosis. The nerves nearby were protected carefully and the adventitia was reserved temporarily.

Harvest of grafts

Cut the skin 1 cm below the inguinal ligament and 1 cm medial to the most obvious place of femoral artery pulse to expose and dissect 8 to 10 cm of femoral veins. Branches of femoral veins were ligated, coagulated and cut off. After thorough dissection of femoral veins, ligate the distal part with 1 to 0 sutures and then the proximal end after emptying the blood flow. Cut the vein at 3 mm proximal to the proximal end and 3 mm distal to the distal end separately. The vein should be handled carefully to prevent mechanical trauma andvasospasm. Distend the vein with heparinized salineto lookfor overlooked tributaries. Then work the graft between the index finger and thumb to overcome the spasm in the vein. Then unligated branches will be secured and reinforced (Sia et al., 2013).

Vascular anastomosis

The right common carotid-left external carotid bypass was operated first. With presence of venous valves, the grafts were put inversely along the blood flow. Then the adventitia at the ends was trimed. The external carotid at the anastomosis between the ingual artery and internal carotid was dissected and then a vascular cushion was put under it. A 4 mm oval incision was made with smooth edges on the external carotid after occlusion of both ends with temporary clamps. Repeat flushing the mural thrombus with heparinized saline. The proximal end of femoral vein was anastomosed with the end of external carotid with interrupted or continuous suture using 8 to 0 micro sutures. Before the last two stitches, heparinized saline was injected to eliminate air inside the artery. During the process of anastomosis, saline was dripped continuously to the surface of vessels for moisture. After anastomosis, the graft was occluded first and the clamps at the distal end of external carotid were released for vascular filling. No exudation was observed and then the clamps at the proximal end of external carotid were released. The total duration of occlusion should not excess 30 mins. No or little exudation was observed and resolved easily by compression. The graft was anastomosed proximally (distal end of the femoral vein to the end of the contralateral common carotid) through a subcutaneous tunnel made with clamps anterior on the front of the neck. The external carotid proximal to the distal end of the anastomosis was ligated. According to the above method, left common carotid-right external carotid bypass was completed and the external carotid was ligated. Finally, the blood flows of the external carotids on both sides were supplied by the contralateral common carotids through grafts (Figure 1C and D).

Post-operative treatment

After the dog restored normal autonomous respiration, the tracheal catheter was removed after consciousness recovery. Dogs were in fasting condition but had free access to water on the day of surgery. General diet was given on the first post-operative day and aspirin started to be administered at 300 mg qd for lifetime.

Measurements and specimen collection

Occlusion time of vessels was recorded during the operation. DSA and color Doppler sonosound examinations were taken 1, 4 and 24 weeks after the surgery according to the group. During the examination, measure and record hemodynamic parameters of grafts. Grafts were taken out 24 weeks after the surgery for histopathological examination.

Statistical analysis

All measurement data would be expressed with $\bar{X} \pm s$ and analyzed with SASS software (SASS version 9.0, SASS Inc.).

RESULTS

Six dog (12 sides) were operated high-volume common carotid-external carotid bypass under a microscope for model building.

General condition of the dogs

The surgeries were successful with no anesthetic accident. All grafts were patent. The operating time was 5 to 8 h. All dogs were of similar mental state, physical activities, foraging and feeding state with before except for one dog that had choking induced by nervuslaryn geuscranialis injury during the surgery.

Vessel occlusion time

Interrupted suture and continuous suture were performed for No. 1 to 2 dogs (n=8) and No. 3 to 6 dogs (n=16), respectively. Continuous suture took less time than interrupted suture (P<0.05) (Table 1).

DSA

DSA on bilateral external carotids showed blood flow provided by the contralateral common carotid through the grafts and patent anastomotic ends and grafts 1 week (n=4), 4 weeks (n=4) and 24 weeks (n=4) post-operatively (Figure 2A and B).

Color Doppler sonosound

Clear Doppler pictures were taken 1, 4 and 24 weeks post-operatively (Figure 2C to D) for measurements of inner diameter and hemodynamic parameters of grafts (Table 2).

Histopathological

Histopathological examination taken 24 weeks after the operations showed intimal hyperplasia and arterialization

Table 1. Vessel occlusion time with interrupted suture and continuous suture.

Group	Number	Vessel occlusion time (min)
Interrupted suture	8	36.25±5.18
Continuous suture	16	20.81±5.27

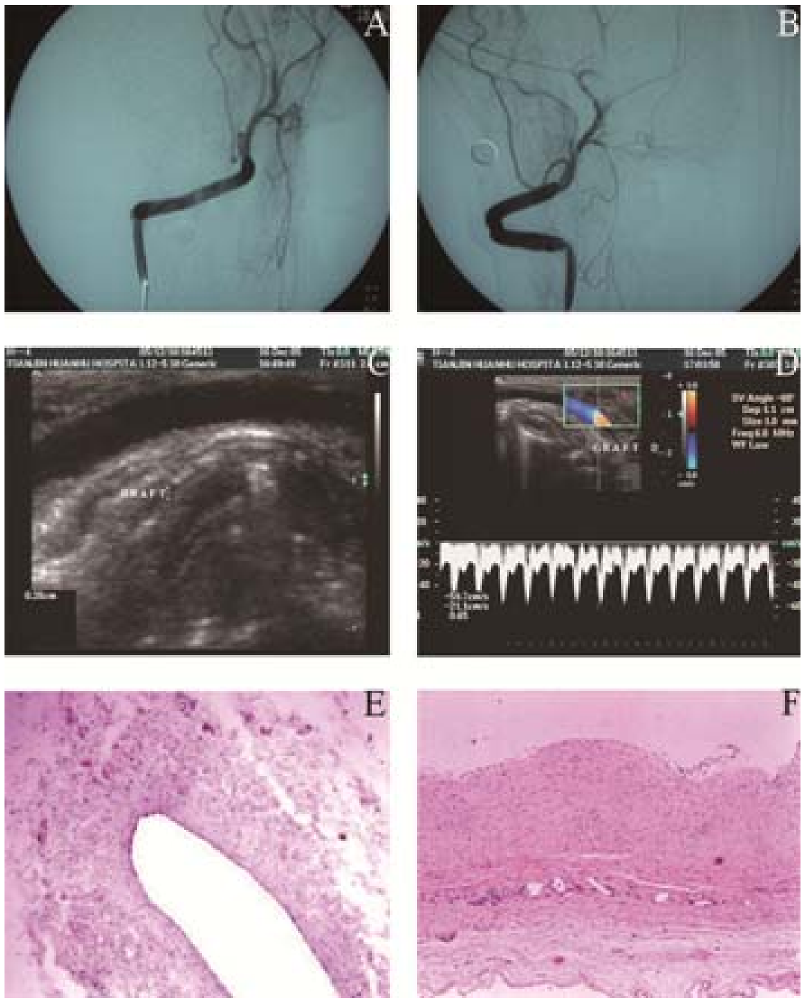

Figure 2. (A) and (B) shows blood flow from the right common carotid to the left external carotid through the graft on DSA. (C)and(D) shows Co-lor Dopper sonosound pictures of cervical vessels (C The graft. D Hemo-dynamic measurements of the graft.). (E) and (F) shows histopathological examinations (E Normal femoral vein of dogs. F Intimal hyperplasia of the graft 24 weeks post-operatively).

Table 2. Inner diameter and hemodynamic measurements of grafts.

Table	Vs (cm/s)	Ved (cm/s)	PI	D (mm)	Q (ml/min)
1-week group	82.8±17.0	24.6±3.2	1.29±0.14	2.75±0.05	205.9±43.9
4-week group	87.7±6.9	21.5±1.9	1.51±0.16	2.50±0.08	194.7±22.5
24-week group	80.9±7.0	21.7±0.7	1.42±0.10	2.57±0.10	184.9±24.8

Vs, peak systolic velocity; Ved, end-diastolic velocity; PI, pulsatility index; D, diameter; Q, blood flow quantity.

of grafts compared to pre-operative veins in the dog model of high-volume common carotid-external carotid bypass (Figure 2E to F).

DISCUSSION

In 1953, saphenous vein bypass was firstly applied by Conley, a head and neck surgeon, in a surgery of a patient with tumor invasion of internal carotid (Conley, 1953). Afterwards, high-volume bypass operations have been improved by various surgeons and widely used. In 1971, Lougheed, a Canadian neurosurgeon and his colleagues described common carotid-internal carotid bypass with saphenous veins (Lougheed et al., 1971). Then (Sundt Jr et al., 1986; Sundt 3rd and Sundt Jr, 1987) improved this technique by using the M2 segment of middle cerebral artery and the P2 segment of posterior cerebral artery as the receiving vessels.

In late 1980s, Sekhar, Spetzler and their colleagues (Sekhar et al., 1987, 1990; Spetzler et al., 1990) reported a technique of intracavernous internal carotid artery reconstruction by direct bypass with saphenous veins. The technique of employing radial artery as a coronary artery bypass graft was firstly induced in intracranial bypass by (Ausman et al., 1978).These years, GiulianoMaselli and his colleagues have reported the possibility of endovascular stent in the event of a narrowing of the bypass (Maselli et al., 2011).

Over the years, with application of intra-operative DSA, improvement in neurosurgical skills and more appropriate choice of patients, high-volume intra-and extra cranial artery bypass has become an important treatment for various intracranial diseases gradually (Eliason et al., 2002; Evans et al., 2004; Deshmukh et al., 2005; Mohit et al., 2007; Sekhar et al., 2008; Patel et al., 2010; Xu et al., 2011; Ramanathan et al., 2012; Kalani et al., 2013). However, high-volume bypass surgery is not an easy-to-master technique, requiring special training. Trainees should first practice suture with gloves, mice and cadavers in labs, read books, literature and see videos as reference and assist experienced surgeons in surgeries (Sekhar and Kalavakonda, 2002). Only through these trainings can surgeons improve knowledge and operation ability for this technique. Nevertheless, these trainings are still of quite large difference with actual practices. For example, the suture feeling of non-biological materials is of great difference with that of mammal vessels. Blood flow volume of small animals cannot meet the demand of high-volume vascular anastomosis. In training with the head of cadavers, hemodynamic measurements could not be monitored. For better surgical results, we chose dogs to build an animal model of high-volume bypass because the internal diameter of canine vessels is close to that of human vessels. This model can resolve the problem of different suture feelings with non-biological materials, meet the volume demand in high-volume bypass and monitor hemodynamic measurements simultaneously.

In the pilot experiment, we found the internal carotid of dogs was too small (about 1.5 mm) to be used in high-volume bypass while the external carotid had a much bigger internal diameter on DSA. External carotid is more vital than internal carotid, it in combination with vertebrovasilar artery provides intracranial blood flow, in consistent with the observed results by (Jung et al., 1975). There are many anastomotic branches between internal and external carotid making longer occlusion of carotid more endurable. For the above reasons, we chose canine common carotid and external carotid as the blood donor and acceptor in the model of high-volume bypass, respectively.

Though saphenous vein is commonly used for “high-flow” extra cranial–intracranial bypass on human, we foundit is not suitable for such surgery on a dog.Canine saphenous vein is much thinner compared with that of humans and the drainage is incorporated into the femoral vein at the middle part of femoral region. The diameter and anatomical position are not fit for grafts. However, Canine femoral vein is larger and more suitable forgrafts in diameter. Besides, the deep femoral vein drainage is incorporated directly into the eternal iliac vein above the inguinal ligament thus lower-limb deep vein drainage through the deep femoral vein will be observed after ligation of the femoral vein. It is safe to remove of femoral vein as the graft.So canine femoral vein was used as the graft in our study.

Canine carotid systemused in experiments has been reported (Crowell and Yasargil, 1973). Once performed end-to-end anastomosis with canine superficial temporal artery and a branch of middle cerebral artery (Asari et al., 1976). Performed anastomosis with the biggest branch of canine external artery, that is maxillary artery and the middle cerebral artery to build an animal model of extra-and intracranial artery anastomosis. Utilization of canine external carotid in high-volume bypass has not been reported yet. The difficulties in building a dog model of high-volume common carotid-external carotid bypass lie in difficult control of anesthesia and high demand for microsurgery. Some experience in our study is discussed subsequently

Vascular dissection technique

Vascular micro dissection technique is the key to success of high-volume bypass. Blood vessels should be dissected with care in avoidance of branch rupture obscuring the operation field before performing the bypass procedures. At the internal carotid root on common carotid, we often encountered a cluster of nerves and vessels, including anterior cervical ganglion, nodosum ganglion, vagosympathetic trunk, and sinus nerve, pharyngeal branch of vagus nerve, nervus laryngeus cranialis, recurrent laryngeal nerve, glossopharyngeal nerve and hypoglossal nerve. Rough dissection might injure these structures causing various symptoms accordingly. The most commonly seen symptom was choking cough and refusal of drinking induced by injuring the nervus laryngeus cranialis, which would cause surgery failure or death. We used rubber band to pull the neurovascular bundle during operation to protect nerves and vessels and provide necessary space for the surgery in the mean while.

Harvest of grafts

As stated above, we chose the femoral veins as grafts. The hind legs in abduction and external rotation would provide a better operation field for surgeons. The position of femoral vein was ascertained medial to the point of maximum impulse in the femoral artery. The femoral vein is located superficially under the skin but it goes backward at the knee into the popliteal vein covered with muscles, causing difficulty in operation. In order to harvest grafts with enough lengths, the incision should be made superiorly. Branches of the graft should be ligated or fully electro-coagulated at 1mm from the trunk. More distal or proximal ligation will have negative effects. We gained satisfying results by using self-made vessel flusher (with a trocar and a 5 ml syringe) to flush the removed grafts with heparinized saline and examine the presence of any leakage.

Anastomosis technique

The opening shapes of common carotid and external carotid are important determinants of post-operative anastomosis stenosis. We employed linear incision at the beginning, which resulted in varying degrees of anastomosis stenosis possibly induced by scar contraction at the incision. Later oval incision was employed and the incidence of anastomosis stenosis was greatly reduced. Anastomosis technique is also a key to the success of high-volume bypass. Too spacing stitches might cause leakage or breakage, while too dense stitches might induce thrombosis or anastomosis stenosis. Veins usually have thinner walls, so wider margin could be adopted. The stitch length was 0.3 mm and the margin was 0.2 mm in our study. The ends of grafts were trimmed with a slope of 45°, thus the anastomosis angle would be 45° along the blood flow. Veins would extend a little under arterial blood pressure, so the anastomosis should be performed under certain tension. Before the anastomosis, the adventitia should be trimmed and eversion suture is recommended to avoid the exposure of adventitia in the lumen. We used 8 to 0 monofilament nylon suture with a length of 7 cm for the anastomosis. Longer suture would affect the operation while too short suture is not favored for knot tying. Three knots should be made and a 1-mm tail is preferred in prevention of slipping. Signs of success are good vascular filling after releasing the clamps, obvious impulse and no leakage or little leakage which could easily stopped with compression for 1 to 2 mins. During the process of anastomosis, continuous suture needed less knot tying and shorter vascular occlusion time (36.25±5.18 min) than interrupted suture (20.18±5.27 min) ($P<0.05$). However, compared to continuous suture, the interrupted suture of the bypass still has its advantages. Firstly, the interrupted suture can avoid the purse string and puckering effects associatedwith the continuous suture. Secondly, there is greater physiological compliance and fewer disturbances to the flow waveform in interrupted anastomoses. (Ferroli et al., 2007)

Conclusions

The results demonstrated a successful animal model by using autologous femoral vein through subcutaneous tunnel to perform common carotid-external carotid bypass leaving the dogs in good health after the surgeries. DSA showed the grafts were patent, which was confirmed with volume above 90 ml/min by color Doppler sonosound.

The histopathological examination showed intima hyperplasia and arterialization and good coverage of endothelium at the anastomosis. All above results suggested the model could be used for further study and application.

In conclusion, this study showed the model of common carotid-external carotid bypass using autologous femoral vein through subcutaneous tunnel was safe and effective. Many factors could affect the successful building of the model, in which improvement of microsurgical skills and anastomosis techniques played a key role.

Conflict of Interest

The author(s) have not declared any conflict of interest.

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