Experience of Vein Grafting in Göttingen Minipigs

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Abstract: We experimented with vein grafting surgery on Göttingen minipigs. Using the internal jugular vein for the tissue graft, we performed side-to-side anastomosis to the carotid artery, to which it runs parallel. One key point in this surgery was to prevent vasospasm of the carotid artery so as to keep the lumen sufficiently patent during anastomosis. The histopathological findings in the grafts which remained patent resembled those of vein grafts in humans. We therefore considered that this technique in minipigs can be applied for the study of coronary artery bypass surgery in humans.

Key words: animal model, minipigs, vein grafting

In the surgical construction of a human coronary artery bypass, the great saphenous vein has been the main vein used as an unpedicled graft, and the internal thoracic artery as a pedicled arterial graft. In general, vein grafting has been less satisfactory in terms of long-term patency than arterial grafting [10], and the causes for this difference have been investigated in a variety of animal models [2, 3, 6, 8, 9]. In these models, the saphenous vein or external jugular vein was used for the graft vein, and end-to-end anastomosis to the carotid artery was the major technique used. Angelini et al. [1] and Toner et al. [12] also made a vein grafting model in domestic pigs with the same technique. We therefore tried to reproduce such a model in Göttingen minipigs. However, the saphenous and the external jugular veins of Göttingen minipigs are very thin and could not maintain a cylindrical lumen because of the shortening just after harvest. Moreover, even though we constructed a vein graft to the carotid artery by end-to-end anastomosis, excessive tension might have been imposed on the grafted vein by its obvious difference of vascular elasticity from the artery. Accordingly, we focused on the internal jugular vein, which runs parallel to the carotid artery, then examined the possibility of a vein graft with side-to-side anastomosis to the carotid artery.

Eight male Göttingen minipigs (Miniature Swine/CSK, CSK Research Park, Inc.) 8 to 10 months of age (body weight: 21.0–26.2 kg) were used in this study. They were kept in an air-conditioned room maintained at a temperature of 22 ± 2°C and a humidity of 50–60% with 12 hr of artificial light (7:00–19:00). They were housed in individual stainless steel cages (65 × 65 × 90 cm) and fed commercial diet (Nisseiken Co., Ltd.) 500 g/day at 9:00 and tap water ad libitum. All animals used in this study were treated in accordance with

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After being fasted for 24 hr, the minipigs were injected intramuscularly with atropine sulfate (Tanabe Seiyaku Co., Ltd.) at a dose of 0.05 mg/kg and azaperone (Stresnil, Sankyo Co., Ltd.) at a dose of 8 mg/kg, and 10 min later received ketamine hydrochloride (Ketalar, Sankyo Co., Ltd.) intramuscularly at a dose of 10 mg/kg. After they had been well sedated, pentobarbital sodium (Nembutal, Dainabot Co., Ltd.)

Fig. 1. Procedure of vein grafting surgery. 1: Internal jugular vein (upper vessel) and carotid artery (lower vessel) after removal of the surrounding connective tissues. The right ends of these portions of vessels are the caudal ends, and the left ends are the cranial ends. 2: Internal jugular vein after its direction was reversed. 3: Side-to-side anastomotic suture between the internal jugular vein and the carotid artery. A stay suture (arrows) has been set up to draw the vein upwards. 4: Internal jugular vein graft just after reperfusion of the blood. 5: Completed vein graft. 6: Schema of the grafted vein and the sites of measurements.
was injected into an auricular vein at a dose of 5–10 mg/kg. When loss of the laryngeal reflex was complete, the minipigs were placed in the supine position on an operating table covered with an electric blanket. Endotracheal intubation was performed with a tracheal tube 8.0 mm in outer diameter. Inhalation anesthesia (O2:N2O=1:2, containing 1.5–3.0% isoflurane (Forane, Dainabot Co., Ltd.)) was induced at a tidal volume of 10 ml/kg and a frequency of 20–25 times/min with a respirator. The condition of the animals was checked by monitoring ECG, blood pressure and body temperature. Until the operation was completed, lactated Ringer’s solution (Solulact, Terumo Co., Ltd.) was infused bilaterally into the auricular veins at a rate of 10 ml/kg/hr via 20-G indwelling needles. The muscle relaxant pancuronium bromide (Myoblock, Sankyo Co., Ltd.) was injected at an initial dose of 0.2 mg/kg and then at a dose of 0.1 mg/kg every 30–60 min; and also the antibiotic (Procaine penicillin G, Nippon Zenyaku Kogyo Co., Ltd.) was injected at a dose of 10,000 units/kg before the operation commenced.

After the anterior side of the neck was shaved and thoroughly disinfected, the skin was incised along the median line from the cartilages of the larynx to the upper end of the manubrium sterni. Both the carotid artery and the internal jugular vein were exposed by removing the surrounding connective tissue (Fig. 1-1). Then, the internal jugular vein was temporarily clamped proximally with bulldog forceps and the presence of a venous valve was confirmed. Since vasospasm of the carotid artery was often observed during these procedures, the rate of infusion of lactated Ringer’s solution was doubled (to 20 ml/kg/hr), and also, a piece of gauze soaked in papaverine hydrochloride (Dainippon Pharmaceutical Co., Ltd.) diluted 1:10 with saline was placed around the vessel to effect dilatation. In order to prevent blood coagulation, a bolus injection of 100 units/kg of heparin sodium (Takeda Chemical Industries, Ltd.) was given, and later, infusion of heparin sodium was initiated at the rate of 15 units/kg/hr. After the carotid artery recovered its normal diameter, both of its ends and both ends of the jugular vein were clamped with bulldog forceps and anastomosis construction surgery was started. When venous valves were observed in the jugular vein, the tip of a cannula (3.3 mm in diameter) was inserted into the cranial end of the internal jugular vein (3–5 mm in depth) just before anastomosis. Then, 0.5–1.0 ml of saline containing heparin was infused into it to bring about dilatation, and double ligation with surgical silk was performed at each end. After measurement of the distance between the two ligated sites on the jugular vein, the vein was transected and inverted so that its cranial and caudal ends were reversed (Fig. 1-2). Anastomosis surgery was performed first at the cranial end, then at the caudal end. In order to construct the anastomoses, the lateral walls of the carotid artery and the jugular vein were partly incised with a scalpel (No. 11) and Metzenbaum scissors. The aperture thus made in the internal jugular vein was drawn with a stay suture up and away from the artery and good patency was maintained (Fig. 1-3). A side-to-side anastomosis was constructed by continuous suturing with atraumatic needles of 7-0 propylene. Both
cranial and caudal ends were declamped immediately after completion of the anastomotic surgery, restoring the blood flow (Fig. 1-4). Finally, the artery bypassed by means of the grafted vein was occluded by ligation with surgical silk to cause all the blood to flow through the venous graft. The carotid artery was never transected, and so excessive tension on the grafted vein was avoided, but the blood contained in the artery was drained through a small incision (Fig. 1-5). Immediately after completion of the vein grafting operation on both carotid arteries, the infusion of heparin was stopped, and the pigs continued to be infused with lactated Ringer’s solution at the former rate (10 ml/kg/hr). After reconfirming the absence of bleeding at the anastomotic sites, the incised skin was closed with a mattress suture and then iodine jelly (Isodine gel, Meiji Seika Co., Ltd.) was applied to the suture site. Immediately after completion of the anastomosis surgery, the supply of isoflurane and N₂O was stopped and only the O₂ supply was continued until respiration resumed spontaneously. After that, the tracheal tube was removed and the minipigs were rehoused in their cages. For analgesia, each minipig was administered flunixin (Banamin, Dainippon Pharmaceutical Co., Ltd.) at a dose of 2 mg/kg for 3 days after the operation. Disinfection of the suture site with iodine jelly was performed every day. All the sutures were extracted 7 days after the operation. During the grafting surgery, the minipigs were observed anatomically and the length of the grafted vein (Fig. 1-6) was measured at the time of completion of anastomosis surgery. As short- and long-term models, one of the pigs and the remaining seven pigs, respectively, were necropsied 24 hr and 16 weeks after anastomotic surgery. Before euthanasia was performed for necropsy, gross examination and manipulation of the grafted veins were performed with the animals unconscious under isoflurane inhalation anaesthesia.

The anatomical observation revealed that only one of the minipigs used in this study had no venous valves in the left or right internal jugular vein. Of the remaining seven minipigs, three had venous valves in one jugular vein, and four, in both. The number of venous valves ranged from 1 to 3 valves/side. The lengths of the grafted veins were 4.7 ± 1.3 cm (2.9–6.9 cm, outer) and 3.4 ± 1.1 cm (1.7–5.5 cm, inner) (Fig. 1-6). The length of the grafted vein depended on number of the venous valves.

In the necropsy findings of the minipig surviving as a short-term model, no abnormal finding was observed. Among the long-term models, only one case of the seven (14.2%) had a bilateral obstruction, but three cases of the seven were patent unilaterally (42.9%), and three, bilaterally (42.9%). Obstruction of the grafted vein seemed to form in animals whose carotid artery showed poor enlargement in the diameter in the early stage of the study. The key point of this surgery was the prevention of vasospasm of the carotid artery to keep the aperture sufficiently open during anastomosis.

In the pathological examinations of the patent grafts in both of the short- and long-term models, no abnormal finding was observed at the anastomotic sites. The inner surface of the grafted vein in the long-term models was found to be covered with a single layer of endothelium, and the vein formed a smooth union with the carotid artery. It was also seen that propagation of smooth muscle cells in the tunica media of the grafted vein occurred to a similar degree to that in the carotid artery (Fig. 2). These histological findings resembled those of vein grafts in humans [5]. We therefore considered that vein graft construction with side-to-side anastomosis to the carotid artery using the internal jugular vein was appropriate for vein grafting models in Göttingen minipigs.

Since mature domestic pigs weigh well over 100 kg, infant pigs aged three months or less and weighing up to 25 kg have been the most common choice for experiments. Therefore, their physiological state is not necessarily good for providing the appropriate conditions for studying human geriatric diseases. On the other hand, Göttingen minipigs have been improved to reduce their body weight at two years of age to 35–40 kg [4]. Therefore, sexually mature and older minipigs provide animal models that are preferable to domestic pig models for the study of diseases of the elderly.

This report describes a novel technique of vein grafting in Göttingen minipigs. By combination with various experimental minipig models already established, including hyperlipidemia and arteriosclerosis models [7, 11], it may be possible to create a minipig model suitable for analysis of the causative mechanism of the occlusion that sometimes occurs in grafted veins after bypass grafting in humans.
# References