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Andrew C. Gordon
Margaret J. Dallman
Peter J. Morris

Vascular anastomotic techniques for experimental intestinal transplantation

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A. C. Gordon (✉) · M. J. Dallman
P. J. Morris
Nuffield Department of Surgery, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DU, UK

Abstract A comparison of two techniques for the vascular anastomosis of intestinal transplants in the rat suggests that the use of an aortic segment with the graft leads to reduced operative time and improved technical success.

Key words Intestinal transplantation, vascular anastomosis, rat · Vascular anastomosis, intestinal transplantation, rat · Rat, intestinal transplantation, vascular anastomosis

Introduction

Since the first description of intestinal transplantation in small laboratory animals [7], the majority of investigators have used the operative technique described by Monchik and Russell. The graft is harvested on a vascular pedicle composed of a segment of aorta from which the superior mesenteric artery arises and the portal vein, which is divided at the porta hepatis. The vascular connections to the host involve microvascular anastomoses between (1) the donor aortic segment and the recipient abdominal aorta and (2) the donor portal vein and the recipient inferior vena cava. Monchik and Russell reported a technical failure rate of 30.4 % in their original series, and recent reports have indicated failure rates of 10 %–15 % at 48 h [1, 4] and 5 days [6]. Two alterations to this technique have been described with various claims as to the relative advantages of the modifications.

Wallander et al. described a technique in which the graft is harvested in the same manner as described by Monchik and Russell, but the vascular anastomoses were constructed by the removal of the recipient's left kidney and the formation of a "cuff" anastomosis between (1) the donor aorta and the recipient renal artery, and (2) the donor portal vein and the recipient renal vein [11]. This resulted in a 5-day survival of 92 % for semi-allogeneic grafts and 98 % for syngeneic grafts, although an additional 18 % of the grafts failed overall as a result of "reabsorption or encapsulation". It seems likely that chronic ischaemia of the graft was responsible for these longer-term failures since blood flow in the graft was lower than in the animal's native gut (as low as 33 % of the flow in the native gut). Additionally, an earlier paper by the same group quoted a 60 % technical success rate postoperatively in more than 150 operations with up to 10 % of the animals dying peroperatively [10].

The second major alteration to the standard technique was described by Harmel, in which the graft is harvested on a vascular pedicle composed of superior mesenteric artery and vein, the dissection not being carried up onto the aorta and portal vein. A shorter segment of gut is therefore harvested on much smaller vessels than previously described. This method was reported in a small number of transplants (n=13), and the technical success rate in syngeneic recipients was just over 50 % after the 1st few postoperative days. The principal causes of failure were anastomotic thrombosis and bleeding [2]. Great importance was placed on the maintenance of adequate intravenous hydration and normal temperature of the recipient during the operation, such that an intravenous cannula was inserted into the internal jugular vein of the recipient prior to operation. Subsequent reports of this method using orthotopic placement of the graft in a fully allogeneic model [3] and a syngeneic model [9] contained no specific reference to technical failure rates, but implied that no technical failures were

Table 1 Operative criteria comparing the two different anastomotic techniques

	SMA-to-aorta (n=50)	Aorta-to-aorta (n=50)	Significance (P)
Technical success	24 (48 %)	33 (66 %)	0.01[a]
Technical failure	26 (52 %)	17 (34 %)	0.01[a]
Average donor weight	253 g	259 g	NS[b]
Average recipient weight	260 g	288 g	0.003[b]
Average time for donor operation	42 min	36 min	0.0002[b]
Average time for recipient operation	76 min	71 min	NS[b]
Cold ischaemia time	22 min	24 min	NS[b]
Warm ischaemia time	43 min	37 min	0.0001[b]

[a] Chi^2 test
[b] Student's t-test

seen in a total of 24 transplants. However, in a further report of this method, at the time of operation three recipients were noted to have developed occlusion of the venous anastomosis (one died peroperatively), and the technical success rate at 24 h was 73 %, with an overall technical success rate of 36.4 % [8]. A similar method was described by Kort et al., who formed the anastomoses with continuous 7/0 nylon sutures and placed the graft in an orthotopic position with the venous drainage directed into the portal circulation. The operative mortality within 48 h was 32.5 % of a total of 40 transplant recipients. The principal cause of technical failure was portal vein thrombosis, which may be largely attributed to the greater technical difficulty of performing an anastomosis to the portal vein [5].

This paper compares the method of Monchik and Russell with that described by Harmel.

Materials and methods

Eight to twelve-week-old male DA and Lewis rats were obtained from Harlan Olac, Bicester, UK, and maintained in the Biomedical Services Unit of the John Radcliffe Hospital where they were fed a standard rat diet with free access to water.

Two sequential groups were compared:

Group 1: Fifty transplants were performed using a slight modification of the technique of Monchik and Russell.

Group 2: Fifty transplants were performed following the method described by Harmel.

For allogeneic transplantation, DA rats were used as donors and Lewis rats as recipients. For syngeneic transplants, DA rats were used as both donors and recipients. Animals were anaesthetised with ether, followed by an intraperitoneal injection of 0.2 mg midazolam hydrochloride and 0.063 mg fentanyl citrate + 2 mg fluanisone. The abdomen was shaved and cleaned with chlorhexidine in spirit.

The operative technique for group 1 was essentially that described by Monchik and Russell. After mobilisation of the small intestine, the graft was isolated on a pedicle of aorta and portal vein. The portal vein was divided just distal to the confluence of the superior mesenteric and splenic veins. The vascular lumen of the graft was not flushed with preservative solution, so the donor animal was heparinised before the graft was removed. Immediately after removal of the graft, the lumen was flushed with 0.9 % saline at 4 °C to remove luminal content and then stored in saline at 4 °C. The donor aorta and the portal vein were anastomosed end-to-side to the recipient aorta and the inferior vena cava, respectively, and the vascular anastomoses were constructed with a continuous single layer of 8/0 nylon sutures for both the artery and the vein.

For the animals in group 2, the operative technique was that described by Harmel, except that the vasculature of the graft was not flushed. After heparinisation of the donor, the graft was removed on a pedicle of superior mesenteric artery and vein that were anastomosed to the recipient aorta and inferior vena cava, respectively. The lumen of the graft was flushed with 0.9 % saline at 4 °C to remove luminal content and then stored in saline at 4 °C. Both anastomoses were performed with a single layer of interrupted 10/0 nylon sutures using 6 sutures for the arterial anastomosis rather than the 12 described by Harmel.

The ends of the transplanted intestinal segment were brought out to the skin as stomata in both groups, the recipient's intestine remaining intact. Postoperative management was identical for both groups. The lumen of the transplanted gut was flushed gently with sterile saline each day to remove accumulated mucus and debris, and the animals were examined for the presence of an abdominal mass, this being the endpoint for diagnosis of rejection in the recipients.

Technical success was defined as survival of the recipient in good health beyond the 5th postoperative day, or until sacrifice of the healthy animal for studies of the graft.

Results

In each group there were 31 allogeneic transplants and 19 syngeneic transplants. There was no significant difference between the weight of the donor animals in the two groups, but the recipients in the aortic group were heavier than in the superior mesenteric artery group. Subjectively, the operation was found to be easier in lighter animals. The donor procedure was very significantly quicker in the aortic group – a rather surprising finding. Although the overall time taken for the recipient procedure was not significantly different between the groups, the warm ischaemia time was very much less in the aortic group. Importantly, the technical success rate of the aorta-to-aorta method was significantly higher (Table 1). Subjectively, no more difficulty was encountered in removing the graft on an aortic segment.

Bleeding from the arterial anastomosis was much more frequent following anastomosis of the superior mesenteric artery to the aorta and was the only cause of technical failure that was significantly different between the two groups (Table 2). Arterial anastomotic thrombosis was seen with equal frequency in each group, but

Table 2 Causes of technical failure in 100 intestinal transplants, comparing the two different anastomotic methods

	SMA-to-aorta (n=50)	Aorta-to-aorta (n=50)	Significance (P)
Anaesthetic	2	0	NS[b]
Anastomotic bleeding	9	1	0.02[a]
Arterial thrombosis	2	2	NS[b]
Venous thrombosis	5	2	NS[b]
Sepsis	2	3	NS[b]
Graft perforation	0	3	NS[b]
Unknown	6	6	NS[b]

[a] Chi^2 test with Yate's correction
[b] Fischer's exact test

there was a tendency towards an increased number of venous thromboses in group 2, although this did not reach significance. The cause of the large number of technical failures, representing animals that died for no apparent reason and that all underwent full postmortem examinations (including histological examination in many cases), was unknown. This problem was seen equally in the two groups.

No syngeneic recipients developed an intra-abdominal mass. There were no significant differences in the period before detection of an intra-abdominal mass between the two different anastomotic groups (Mann-Whitney U-test). A comparison of body weight reveals that both syngeneic and allogeneic recipients lost weight after transplantation, but there was no significant difference when the two different anastomotic methods were compared in either syngeneic or allogeneic recipients (Mann-Whitney U-test). Similarly, there were no significant differences between the two anastomotic methods in terms of the survival of allogeneic or syngeneic recipients (Mann-Whitney U-test).

Discussion

Although the two groups of animals were consecutive and not randomised, the series of operations in group 1 was begun after the general principles of the procedure had been learnt by an initial series of 24 successful operations, all of which had been performed by the operative technique used in group 1.

When successful, both anastomotic techniques resulted in a well-vascularised transplant of the entire small intestine. Technically, it was found to be much easier to perform an anastomosis between the graft aorta and the recipient aorta than between the graft superior mesenteric artery and the recipient aorta because of the size of the vessels. Attempts to use a continuous suture for the latter anastomoses were unsuccessful mainly because of a tendency for the anastomosis to become stenosed when the suture was tied. It seemed that the increased bleeding problems encountered in the group 2 animals stemmed principally from bleeding between the sutures. This problem may be partly related to using 6 rather than 12 sutures as described by Harmel, but the insertion of double the number of sutures into this small anastomosis would inevitably increase the time taken even further, with no obvious benefits. The principal problem on the venous side was the tendency for stenosis of the anastomosis due to too small a venotomy being performed. In this regard the larger diameter of the graft vein in group 1 animals gave a greater margin of safety. Although the incidence of technical failure in our series was higher than frequently reported, we have defined and adopted a vigorous definition of technical success.

Our data reveal no significant differences between the two groups in terms of the survival of the recipient, the postoperative weight loss or the time to develop an abdominal mass in the allogeneic recipients. Thus, the recipients seem to behave in a similar manner, whichever anastomotic method is used.

The contention that removing the graft on a pedicle of superior mesenteric artery and vein reduces the operative time "by several hours" [8] is not supported by the results described here, in which the average total operative time in group 1 was 118 min, and in group 2 was 107 min. Indeed, the donor operation was significantly shorter if the graft was harvested on an aortic segment. The extra time needed to remove the graft on a pedicle of superior mesenteric artery is taken up in separating the narrow, thin-walled vessels from each other and from the mesentery. The shorter warm ischaemia time in the aortic group is an important finding since the intestine is extremely sensitive to ischaemic damage.

In conclusion, there is no evidence from the data presented here that harvesting intestinal grafts on a pedicle of superior mesenteric artery rather than aorta offers any benefit in terms of time taken, ease of operation or operative results. In view of the rather conflicting data about the cuff anastomotic technique, there seems little justification for deviating in any major way from the technique described more than 20 years ago by the pioneers of rodent intestinal transplantation [7].

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