Increased intimal hyperplasia in experimental vein graft stenting compared to arterial stenting: comparisons in a new rabbit model of stent injury

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Abstract

Background: In-stent restenosis due to intimal hyperplasia is an important clinical problem. Animal models of stent injury are limited by inconsistent arterial responses to stenting, and less intimal hyperplasia than diseased human vessels. To address these issues, we aimed to compare the degree of intimal hyperplasia in stented rabbit jugular–carotid interposition grafts (vein grafts) versus stented carotid arteries. Methods: Jugular–carotid vein grafts were constructed in rabbits, then stented or left unstented. Carotid arteries were treated with similar stents or left instrumented only. After 3 or 28 days, vessels were perfusion fixed, embedded in resin, and sections were cut with a diamond saw. Intimal and medial thicknesses were measured in stained sections. Results: After 3 days, inflammatory changes were observed in the intima of all stented vessels. After 28 days, intimal thickness in stented vein grafts was 2-fold greater than in control vein grafts and approximately 4-fold greater than in stented carotid arteries. In addition, the intimal hyperplasia response was markedly more consistent in stented vein grafts compared with stented carotid arteries. Conclusions: Stent deployment in experimental vein grafts results in increased and more reproducible smooth muscle cell intimal hyperplasia than carotid arterial stenting. This is a promising small-animal model for investigating the intimal response to stenting.

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1. Introduction

The widespread use of endovascular stents has improved outcomes following percutaneous coronary intervention, but stent restenosis due to excessive intimal hyperplasia remains an important clinical problem. Studies of the biology of intimal hyperplasia in humans are limited because of difficulties obtaining clinical specimens. Animal models may offer important insights into the cellular mechanisms underlying intimal hyperplasia. However, stent injury in healthy animal arteries often results in less intimal hyperplasia than in diseased human vessels, and there may be an inconsistent response to stent injury, leading to reduced statistical power in comparative studies. The biological variation observed may be due to non-uniform stent strut expansion, or to variability in vessel wall penetration and injury. The objective of this study was to develop a small animal model of stent injury with increased intimal hyperplasia compared to stent deployment in native arteries.

Several studies have reported marked intimal hyperplasia in rabbit jugular–carotid interposition vein grafts [1–3], that develops in response to arterial pressure and turbulent blood flow in the graft. During the first 3 days, there is endothelial cell damage with an acute inflammatory reaction, followed by smooth muscle cell proliferation...
and migration [4]. By 28 days an anatomically intact but dysfunctional endothelium has reconstituted, with a mature neointima. This model has been used widely to study the biology of intimal hyperplasia, its response to anti-proliferative drugs [5,6] and as a model for vein graft gene transfer [7,8]. We hypothesized that deployment of a stent from the onset of a vein graft’s exposure to the arterial circulation would lead to enhanced intimal hyperplasia—a response to ‘double injury’ by the stent and by increased blood pressure.

Accordingly, we explored the feasibility of stent deployment in rabbit jugular–carotid vein grafts, and evaluated the intimal response in stented vein grafts compared to both control vein grafts and stented native carotid arteries.

2. Methods

2.1. Animals

This study conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). Male New Zealand White rabbits (2.0–2.5 kg) were given free access to standard rabbit chow and water. From 24 h before surgery until vessel harvest, all rabbits were treated with aspirin (10 mg/kg per day) dissolved in their drinking water.

2.2. Operative procedures

Surgical anesthesia was induced with midazolam (Hypnovel, 2–2.5 mg, Roche Pharmaceuticals) and fentanyl/ fluanisone (Hypnorm, 0.13–0.16 mg/4–5 mg, Janssen Pharmaceuticals) injected subcutaneously. Anesthesia was maintained with inhaled oxygen, nitrous oxide, and halothane (Fluothane, 1–2.5 ppm, Zeneca Pharmaceuticals).

After intravenous heparinisation (300 u/kg), jugular–carotid interposition vein grafts were constructed as previously described [4]. Briefly, for each graft, a 3-cm segment of external jugular vein and ipsilateral common carotid artery were carefully dissected from surrounding tissue, and side branches ligated. The jugular vein segment was excised, reversed, and anastomosed end-to-side across the common carotid artery (arterial blood flow controlled by micro-clips), using 10/0 nylon microsutures (Ethicon, UK). The carotid artery was ligated with 4/0 nylon microsutures (Ethicon, UK) and arterial flow re-established.

To introduce a stent into newly constructed vein grafts, one side of the caudal anastomosis was initially left unsutured, leaving an opening into the lumen of the graft. An 11-mm stainless steel BiodivYsio stent (Biocompatibles, UK) was mounted on a 4.5×13-mm Viva Primo angioplasty balloon (Boston Scientific), introduced into the vein graft, and the balloon was inflated to 12 bar for 30 s (final nominal balloon diameter 4.7 mm). This inflation pressure was found to give optimal stent expansion within the vein graft. The balloon was deflated and withdrawn (Fig. 1a), the caudal anastomosis completed, the carotid artery ligated and divided, and blood flow re-established (Fig. 1b).

Carotid arteries were stented as follows. After heparinisation, a 3-cm mobilized segment of common carotid artery was clipped, and a 2-mm longitudinal arteriotomy was made. An 11-mm stent as above was mounted on a 2.0×13-mm Viva Primo angioplasty balloon, introduced into the carotid artery and the balloon inflated to 14 bar for 30 s (final nominal balloon diameter 2.25 mm), aiming to achieve a stented vessel diameter of 1.3–1.5 times the reference vessel segment. The arteriotomy was repaired with 10/0 nylon sutures, and arterial flow re-established. Control carotid arteries were mobilized, but not stented, to control for the effects of operative instrumentation.

After 3 or 28 days, under general anesthesia, carotid arteries and vein grafts were dissected free, and 300 u/kg heparin was given intravenously. Animals were euthanised with an intravenous overdose of pentobarbital sodium. Vessels were immediately perfusion fixed for 20 min at a pressure of approximately 100 mmHg by infusing 4% paraformaldehyde solution in PBS via a 14G cannula into the upper thoracic aorta, having tied off the distal aorta and superior vena cava. Vessels were harvested and fixed for a further 4 h at 20 °C in 4% paraformaldehyde in PBS.

2.3. Histomorphometric analysis

Fixed vessels were dehydrated in 70% ethanol at 20 °C for 4 h, then in 100% acetone at 4 °C for 1 h. Vessels were embedded in methyl methacrylate resin (Technovit T8100, TAAB Laboratories) for 24 h on ice, according to the manufacturer’s instructions. Sections (5–10 μm) were obtained by cutting embedded vessels using an Isomet 2000 diamond-coated rotary saw and polishing with a Metaserv 2000 grinder/polisher (Buehler, UK), as previously described [9].

Sections were stained with haematoxylin and eosin at 60 °C, as previously described [9]. Intima and media thickness in each vessel was measured using a Leica DMRBE microscope with a ×20 objective; image analysis was performed by video capture and Leica Q500 MC Qwin software. Photomicrographs were taken with a Nikon Coolpix 950 digital camera.

2.4. Statistical analysis

Two perpendicular luminal diameters were measured in three sections per vessel (one from each third of the stented segment), and combined as n=1. Intima and media thickness were assessed at eight points around the circum-
Fig. 1. Operative photographs of jugular-carotid interposition vein graft stenting. (a) The reversed jugular vein segment is anastomosed (end-to-side) to the left carotid artery, with the caudal suture line partially completed (arrow). A BiodivYsio stent (11 mm length) mounted on a 4.5×13-mm angioplasty balloon is introduced into the vein graft via the caudal anastomosis, and the balloon inflated to 12 bar for 30 s. (b) The caudal anastomosis is completed, the intervening carotid artery is ligated and divided, and arterial clips are removed to establish blood flow through the stented vein graft.

ference for three separate sections per vessel, and the mean values used as $n=1$. Means from three vessels were combined to provide overall mean values ($n=3$). Groups were compared using single factor ANOVA. $P$ values <0.05 were considered significant.

3. Results

Operations were performed on a total of 27 rabbits, with bilateral procedures in most cases. One rabbit died under anaesthesia. Five other rabbits died during the first 2 days after surgery, following bilateral stenting. Thereafter, for each rabbit, stents were deployed in either native carotid artery, or vein graft, but not both. Results are reported for vessels from animals that survived to the pre-specified time point.

3.1. Vessel diameters

3.1.1. Carotid arteries

The mean luminal diameter of perfusion fixed non-stented carotid arteries, harvested 3 days after intervention, was 1375 (standard deviation 146) μm, compared to 2394
Table 1  
Vessel luminal diameters after harvesting

<table>
<thead>
<tr>
<th>Time point</th>
<th>Vessel</th>
<th>Mean (S.D.) (µm) (n=3)</th>
<th>Ratio of diameter of stented to non-stented vessel</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 days</td>
<td>Carotid</td>
<td>1375 (146)</td>
<td>1.74</td>
</tr>
<tr>
<td></td>
<td>Carotid stent</td>
<td>2394 (111)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vein graft</td>
<td>4018 (148)</td>
<td>1.23</td>
</tr>
<tr>
<td></td>
<td>Vein graft stent</td>
<td>4930 (93)</td>
<td></td>
</tr>
<tr>
<td>28 days</td>
<td>Carotid</td>
<td>1361 (125)</td>
<td>1.71</td>
</tr>
<tr>
<td></td>
<td>Carotid stent</td>
<td>2334 (101)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vein graft</td>
<td>4318 (327)</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>Vein graft stent</td>
<td>4965 (115)</td>
<td></td>
</tr>
</tbody>
</table>

*S.D., standard deviation.

(111) µm in stented carotid arteries (P<0.001), with a diameter ratio of 1.74 (Table 1). After 28 days, there was no significant change in these values.

3.1.2. Vein grafts

The mean luminal diameter of control vein grafts, harvested 3 days after intervention, was 4018 (148) µm, compared to 4930 (93) µm in stented vein grafts (P<0.001), diameter ratio 1.23. After 28 days, the mean luminal diameter of control vein grafts had increased to 4318 (327) µm (P=0.07), whereas there was no significant increase in the diameter of stented vein grafts (4965 (115) µm) (P=0.57).

3.2. Histological and histomorphometric analysis

Detailed histological descriptions of the vessel sections are given in the legends to Figs. 2 and 3, with key points summarized below.

3.2.1. Three days post intervention

In control carotid arteries that underwent surgical isolation but no stent deployment, there was normal vessel architecture with no inflammatory cells. In stented carotid arteries there was organizing thrombus around stent struts, with inflammatory cell infiltration at sites of vessel injury. In control vein grafts there was partial endothelial denudation, with inflammatory cells in the media and adventitia. In stented vein grafts there was a similar inflammatory reaction in the vessel wall, with additional inflammatory cells localized in areas of organizing thrombus around the stent struts. At this stage, intimal thickening was mainly due to inflammatory cell infiltration, and this was significantly increased in control vein grafts (P=0.039) and in stented vein grafts (P=0.020) compared to stented carotid arteries (Fig. 4a).

3.2.2. Twenty-eight days post intervention

In control carotid arteries, there were no differences in vessel appearance after 28 days compared to vessels harvested after 3 days. In stented carotid arteries the degree of intimal hyperplasia was highly variable between individual animals, ranging from vessels with moderate hyperplasia covering all stent struts, to vessels with almost no mature intimal thickening but a persisting inflammatory infiltrate around the struts. The mean intimal thickness was 39 µm (standard deviation 28 µm) with a high coefficient of variation (CV) of 0.69 (Fig. 4b). In control vein grafts the endothelium had re-constituted, with increased intimal hyperplasia compared to stented carotid arteries (mean 106 (26) µm) (P=0.038), and a more consistent response (CV=0.25) (Fig. 4b). In stented vein grafts, there was increased intimal thickening compared to control vein grafts (mean 196 (30) µm) (P<0.001) with a more consistent response (CV=0.15) (Fig. 4b).

4. Discussion

In this study we developed and evaluated a new model of intimal hyperplasia in response to stent implantation in experimental rabbit jugular–carotid vein grafts. The operative techniques were feasible and the interventions were generally well tolerated. The major findings were:

1. modest intimal hyperplasia in stented carotid arteries after 28 days, with marked variability in the response between animals;
2. increased intimal hyperplasia in control vein grafts compared to stented carotid arteries after 28 days, with more consistent responses between animals;
3. A 2-fold increase in intimal hyperplasia in stented vein grafts compared to control vein grafts after 28 days (and a 4-fold increase compared to stented carotid arteries). The intimal response was markedly more consistent after vein graft stenting than after carotid artery stenting.

The degree of intimal hyperplasia observed at 28 days in control vein grafts was similar to that reported in previous studies [7,8], which confirms the reproducibility of the rabbit jugular–carotid vein graft model, and the validity of our comparative measurements.

Stent deployment within the rabbit jugular–carotid vein graft is a logical development to produce a ‘double-injury’ effect in this well-established model. Previous studies have already described the pathophysiology of intimal hyperplasia in the rabbit jugular–carotid vein graft [1–3], and its increase in response to hypertension or hypercholesterolemia [3]. The intimal response has also been evaluated following treatment with drugs affecting the cellular redox state [10], and the renin–angiotensin system [5]. The feasibility of in vivo gene transfer to vein grafts has been
established [1], and a recent study showed that nNOS gene transfer markedly reduced intimal hyperplasia in rabbit jugular–carotid vein grafts [8]. Thus, the rabbit jugular vein graft provides a well-validated model to study and quantify the intimal response to vessel injury and its modulation by dietary, pharmacological or genetic interventions.

Our findings suggest that this model may be useful to evaluate the biology of intimal hyperplasia in response to both turbulent arterial blood flow and the presence of a stent. The combination of increased intimal hyperplasia compared to control vein grafts and reduced variability compared to carotid artery stenting would increase the statistical power of such studies to detect differences in biological outcome.

4.1. Mechanisms

The relatively limited intimal hyperplasia response we observed in the stented carotid arteries is partly due to the marked variability of this response in these vessels. Some vessels had virtually no intimal hyperplasia after 28 days, which contributed both to the high variability, and the overall low mean intimal thickness in this group. In this model, intimal hyperplasia may be partly initiated by mural thrombus formation during the early days following stent implantation. Thus, variability in early thrombus formation and stability may have contributed to the variability in intimal hyperplasia observed after 28 days. In addition, variation in arterial wall penetration and injury by the stent struts may also have led to differences observed between animals. Indeed, stent deployment in other arterial models such as the pig coronary artery leads to inconsistent responses, depending on the anatomical characteristics of the vessel injury [11,12]. The ‘double injury’ in the vein graft model combines stent injury with an existing stretch injury that is determined by the hemodynamic factors intrinsic to the vein graft model. In contrast to double balloon injury, or arterial overstretch injury alone, the additional injury is less dependent on operator or procedure-related factors, so may lead to a more consistent and uniform response to injury.

Several possible mechanisms may underlie the increased intimal thickening observed in stented vein grafts compared to control vein grafts. The cellular response in rabbit jugular–carotid vein graft of acute inflammation followed by intimal hyperplasia has already been described [1–4]. The presence of a metallic stent from the beginning of vein graft construction may enhance or prolong the inflammatory process, leading to increased intimal hyperplasia by 28 days. In addition, an increased mural thrombus burden provoked by stent implantation could provide a further stimulus to intimal hyperplasia. Early increases in both inflammation and thrombus are indicators of a later increase in intimal hyperplasia in porcine coronary arteries after stent injury [12]. Stented vein grafts had a 1.2-fold increased diameter compared to control vein grafts, so increased wall tension following stent deployment may contribute. Finally, other mechanical factors, such as the splinting of stented vein grafts, reducing their compliance, may affect the vessel wall tension compared to control vein grafts in the setting of pulsatile systemic arterial blood pressure.

4.2. Limitations

We used a single design and size of stent in both carotid arteries and vein grafts to allow for controlled comparisons between these vessels. However, the difference in diameter between carotid arteries and vein grafts resulted in differences in stent strut geometry, which may influence the intimal hyperplastic response [13]. We did not directly measure the perfusion pressure achieved during perfusion fixation of neck vessels in each animal, and this may have led to variability in fixed vessel dimensions. In particular, it is likely that the control carotid arteries did not attain their full physiological diameter during the perfusion fixation process. This may explain why the diameter ratio of stented to control carotid arteries was 1.7 rather than the 1.3–1.5 intended. For obvious reasons, the histomorphometric analysis was not blinded, and this may have introduced bias into the measurements.

It is important to note that this model does not reflect stenting in human saphenous vein coronary artery bypass grafts, because our stents were deployed at the time of vein graft construction, rather than in mature vein grafts. In this study, we intended to evaluate the effect of stents on intimal hyperplasia in de novo vein grafts, aiming to increase the intimal response. However, the model could be developed so that stents are deployed as a second procedure into mature jugular–carotid vein grafts.
after 3 days. (c,d) Stented carotid arteries. The degree of intimal hyperplasia observed was highly variable between individual animals. In some vessels (c) there was moderate intimal hyperplasia up to 100 μm thick. In other vessels (d) there was almost no intimal hyperplasia, but persisting inflammatory cells were accumulated around the luminal surface of the stent struts (white arrow heads). (e,f) Control vein grafts. By 28 days, an anatomically intact endothelium had re-constituted, and consistent intimal hyperplasia approximately 100 μm thick was observed. (g,h) Stented vein grafts. In all stented grafts the endothelium had re-constituted by 28 days. In most grafts, the stent struts were surrounded by marked consistent intimal hyperplasia, approximately 200 μm thick. Some persisting inflammatory cells were seen adjacent to stent struts.

5. Conclusions

We have demonstrated the feasibility of endovascular vein graft stenting in a small animal model. We observed a 2-fold increase in intimal hyperplasia compared to that seen in experimental vein grafts alone, and a 4-fold increase compared to stented carotid arteries. In addition, intimal hyperplasia in stented vein grafts was more reproducible than in stented carotid arteries. Such a combination of marked intimal thickening with reduced biological variability would increase the statistical power of this model to detect differences in intimal hyperplasia in comparative experiments. This is therefore a promising small animal model for evaluating the biological response to stent injury, and therapies designed to modify intimal hyperplasia.

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References


