Response to balloon injury is vascular bed specific
A consequence of de novo vessel structure?

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Abstract

Relative contributions of remodelling and neointimal hyperplasia to restenosis after coronary angioplasty have been inferred from studies using iliofemoral arteries, despite differences in structure/function and smooth muscle cell lineage. We compared the response to balloon overstretch injury of coronary arteries (C, n = 16) and similar sized branches of the iliac arteries (I, n = 18) using preinjury vessel diameter (P), inflated balloon size in vivo (B) and the manufacturer predicted inflated size (M) to examine arterial compliance, as well as resulting injury and morphology in perfusion fixed vessels. Despite similar degrees of oversizing (M/P) in the coronary and iliac arteries (C, 1.44 ± 0.04; I, 1.51 ± 0.02), the compliance to overstretch (B/P : M/P) was significantly greater in the coronary than the iliac arteries (C, 0.71 ± 0.05; I, 0.51 ± 0.03) (P < 0.05) and was associated with a higher injury score (C, 1.64 ± 0.31; I, 0.39 ± 0.18 P < 0.05)—only 5/18 iliac vessels had rupture of the IEL compared with 13/16 in the coronary bed. In a subgroup of animals whose vessels (C:n = 7; I:n = 8) were perfusion fixed 28 days after injury, coronary arteries had greater intimal area (C:1.03 ± 0.42; I:0.10 ± 0.03 mm², P < 0.05) but larger luminal area (C:1.61 ± 0.71; I:0.76 ± 0.51, P < 0.05) due to greater area within EEL (C:3.38 ± 0.49:1.49 ± 0.54, P < 0.05) or less inward remodelling. The injuries resulting from similar strategies of balloon overstretch in the coronary and the iliac arteries are different and affect healing responses-iliac arteries remodel more while coronary arteries develop more intimal hyperplasia. These results indicate that caution is warranted when extrapolating results from the iliac to the coronary artery when investigating restenosis after angioplasty. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Balloon overstretch; Coronary arteries; Iliac arteries

1. Introduction

Restenosis, which remains a significant limitation of percutaneous coronary revascularisation, results from a combination of negative remodelling (reduction in area within the external elastic lamina) and intimal growth (by smooth muscle cell proliferation and extracellular matrix deposition) [1]. Many experimental studies evaluating mechanisms of and therapeutic strategies for restenosis have been performed in the iliofemoral arteries [2,3] despite potential differences from the coronary arteries regarding both of these responses to injury.

The coronary arteries are morphologically distinct from peripheral arteries of similar size and this may affect the extent of injury and intimal hyperplasia after angioplasty. As the coronary vessels form by vasculogenesis (in situ formation by colocation of cellular elements) rather than angiogenesis (axial growth from a vessel bud regulated by haemodynamics) [4], the internal elastic lamina is more fenestrated and incomplete and is more prone to forming intima [5]. In addition, as the coronary arteries are perfused during diastole, optimisation of the pressure-flow relationship does not depend on the propagation of the systolic pressure wave, which requires vessel wall elasticity. Consequently the external elastic laminae is thinner [6], making rupture of the elastic laminae more likely, which has been strongly linked to neointima formation after injury [7,8].
Remodeling responses may also be different to those observed in the coronary arteries. Indeed, negative remodelling is more prevalent in primary atherosclerotic lesions in the iliofemoral bed than in the coronary arteries [9], it is quite possible that negative remodelling would also be more frequent in the iliofemoral arteries after balloon dilatation.

Despite these potential differences a direct comparison of coronary and iliac injury patterns and healing responses has not been performed. We therefore compared the response to balloon overstretch injury of iliofemoral and coronary vessels, by assessing compliance to balloon inflation, and the relative contributions of remodelling and intimal hyperplasia to lumen loss within the arteries during the healing response. We found that iliac arteries were less compliant than coronary vessels to overstretch, had less rupture of the elastic laminae and formed much less neointima, but had reduced lumen size 28 days after injury due to accentuated negative remodelling.

2. Methods

2.1. Animals, study design and drug administration

Mature male Boston mini-pigs (26–40 weeks old, 40–60 kg), were obtained from a colony at Monash University, Clayton, Australia. Injury of four arteries was attempted in each of the nine pigs: two coronary and two iliac branches. In all cases the iliac arteries injured were the right and left recurrent circumflex branch of the external iliac artery which are similar in size to the coronary arteries (2.5–3.0 mm diameter). Two of the three main coronary branches (right coronary, left anterior descending and left circumflex coronary arteries) were dilated in each pig, except in two cases where only one was dilated due to difficulty with catheter engagement or arrhythmias. Angiographic measurements and injury scores were assessed for all vessels (see below). Seven coronary and eight iliac vessels (four pigs) were perfusion fixed in vivo 28 days after initial injury, and embedded in paraffin for morphometric assessment of vessel areas. Ten iliac and nine coronary vessels (five pigs) were harvested fresh at 5, 14 and 28 days, and were embedded in OCT (‘Tissue Tek’, Miles, Elkhart, IN), frozen using isopentane (Unilab, Australia) in liquid nitrogen and then stored at −70°C for immunohistochemical analysis as part of a separate study.

All animals were administered 300 mg Aspirin (Reckitt & Colman, West Ryde, Australia) per day orally, from 7 days prior to the initial procedures until the day of sacrifice, as well as 120 mg slow-release Verapamil (half a scored 240 mg tablet, Knoll, Lane Cove, Australia) within 12 h before each surgery. Immediately prior to surgery the pigs were premedicated with intramuscular Acepromazine (0.1 mg/kg, Delta, Hornsby, Australia), Droleptan (10 mg, Delta, Hornsby, Australia) and Atropine sulphate (1.2 mg, Delta West, Bentley, Australia), anaesthesia induced with intravenous Propofol (150–200 mg, ICI, Melbourne, Australia), and then maintained with inhaled isoflurane (Abbott, Kurnell, Australia).

2.2. Angiography and angioplasty

All procedures were performed using an 8F JL4 guiding catheter through a sheath inserted into the common carotid artery (initial procedure right carotid, follow-up left carotid) after heparinisation (15 000 units, Fisons, Thornleigh, Australia). Angiography was performed after intraarterial glyceryl trinitrate 200 μg (Fisons, Thornleigh, Australia) using Ioxaglate (Hexabrix, Mallinckrodt, Notting Hill, Australia) and was recorded for later analysis (Super VHS tape, Fuji, Germany) in the left anterior oblique view (25°) for the coronary arteries and in straight anteroposterior view for the iliac vessels. To easily identify the injured segment at vessel harvest, the most proximal segment of each artery was injured. Arteries were balloon injured using standard human angioplasty catheters (semiconformant, 20 mm length) which were oversized according to the manufacturer-specified inflated balloon size with balloon:artery ratio of 1.3–1.5:1. The balloon catheter was inflated to 10 atm for 30 s with three separate inflations separated by 1 min reperfusion periods. All measurements of vessels were made using handheld digital calipers (Mitutoyo, Japan) from the cine frame at end diastole at the point where the middle marker of the balloon catheter was sited, and using the guiding catheter as a reference. Vessel compliance (VC) was calculated by the following formula:

\[ VC = (B - P)/(M - P) \]

where \( B \) is inflated balloon size in vivo, \( M \) is manufacturer predicted inflated balloon size and \( P \) is preinjury vessel diameter.

2.3. Perfusion fixation and vessel harvest

The injured segment was readily identifiable at harvest due to thickening of the vessel wall. In those animals whose arteries were used for morphometry, vessel beds were first isolated and then perfusion fixed with 4% formalin (in phosphate buffered saline (PBS) pH 7.4) at 100–150 mm Hg for 5 min. To isolate the recurrent circumflex iliac arteries the distal aorta, internal iliac arteries and the external iliac arteries distal to the recurrent circumflex branch were ligated and the fixative line introduced to the aorta distal to the ligature. To drain the bed an incision was made in the
inferior vena cava. To fix the heart the aorta was cross-clamped and the right and left atrial appendages incised, simultaneous to commencement of fixation through an aortic root cannula. After 5 min in vivo fixation, the vessels were then excised and carefully debrided of the perivascular tissues, stored in 4% formalin in PBS for 24 h prior to paraffin embedding.

2.4. Morphometry and injury score

All vessels were cut into segments of 3 mm length resulting in six to seven sampling sites per balloon injured vessel. The region of maximal injury and intima formation were used for injury score and morphometry respectively (usually the same site).

Injury scores were assessed from 4 μm sections (both frozen and perfusion fixed vessels) in which the elastic laminae were highlighted using Masson’s Trichrome with Orcein staining or Van Giessen–Verhoff’s stain [10] for perfusion fixed and frozen vessels respectively.

Injury score was assigned as previously described [7]:
0, Internal elastic lamina (IEL) not broken
1, IEL broken but media and external elastic laminae (EEL) intact
2, IEL broken with deep medial injury and partial rupture of EEL (EEL damaged but structurally intact)
3, EEL ruptured.

Intimal fracture length ratio (IELf/c) was calculated as the proportion of the IEL circumference which had been disrupted as previously described [8]. Medial, neointimal and vessel areas were determined for the perfusion fixed vessels only by planimetry using customised software (Capricorn Scientific, Woori Yallock, Australia) from sections magnified with a projecting microscope (NeoPromar Leitz Germany) onto a digitising tablet (Complot series 7000 digitiser, Bausch and Lomb, Austin, TX, USA).

2.5. Calculation of late loss due to intima formation and remodelling

An estimate of the proportion of late angiographic loss which could be attributed to intima formation was calculated as follows: lumen assumed to be circular immediately post dilatation and at follow-up; the cross-sectional area of the lumen at these two time points was then calculated as \( \pi r^2 \) or \( \pi \) (minimal lumen diameter/2)\(^2\). The difference between these two areas was then calculated as late area loss. The proportion of late area loss due to intima formation was calculated by dividing intimal area on histology by late area loss. The remainder was then assumed to be due to inward remodelling.

2.6. Statistics

All data are presented as mean ± SEM. For comparison of two categorical variables \( \chi^2 \) test was used or Fisher’s exact test where appropriate. For comparison of a continuous variable in coronary and iliac vessels Students’ t-test was used. For comparison of vessel compliance and balloon oversizing in multiple categories (injury score) analysis of variance was used with post-hoc pairwise testing by Student’s t-test. For univariate regression analysis, simple linear regression was used. For multivariate analysis stepwise linear regression was used.

3. Results

3.1. Relative compliance of the coronary and iliac arteries to overstretched injury and relationship to severity of injury sustained

The mean diameter on baseline angiography (\( D \)) and degree of balloon oversizing (\( M/P \)) of coronary and iliac vessels were similar. However, despite identical inflation pressures and times, expansion of the balloon (or compliance, \( B = P/M \)) within the coronary arteries was significantly greater than that seen within the iliac vessels (Table 1). As resistance to expansion may be significantly reduced if the elastic laminae were ruptured we also performed exploratory comparative analysis of compliance in the subgroups of iliac and coronary vessels where the IEL was intact (injury score 0, \( n = 13 \) iliac, \( n = 3 \) coronary vessels), those where the

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Angiographic measurementsa</th>
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<tbody>
<tr>
<td><strong>Angiographic measurement</strong></td>
<td><strong>Coronary</strong></td>
</tr>
<tr>
<td>Baseline lumen diameter (mm)</td>
<td>2.64 ± 0.05</td>
</tr>
<tr>
<td>Balloon oversizing (( M/P ))</td>
<td>1.51 ± 0.02</td>
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<tr>
<td>Compliance (( B = P/M ))b</td>
<td>0.71 ± 0.05*</td>
</tr>
<tr>
<td></td>
<td>( (n = 16) )</td>
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<tr>
<td>IS 0</td>
<td>0.65 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>( (n = 3) )</td>
</tr>
<tr>
<td>IS&lt;3</td>
<td>0.67 ± 0.06*</td>
</tr>
<tr>
<td></td>
<td>( (n = 12) )</td>
</tr>
<tr>
<td>IS 3</td>
<td>0.87 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>( (n = 4) )</td>
</tr>
<tr>
<td>Lumen diameter post-angioplasty (mm)</td>
<td>2.99 ± 0.12*</td>
</tr>
<tr>
<td>Lumen diameter at follow-up (mm)</td>
<td>2.14 ± 0.18*</td>
</tr>
<tr>
<td>Late lumen loss (mm)</td>
<td>0.85 ± 0.09*</td>
</tr>
</tbody>
</table>

a All values are mean ± SEM. * \( P < 0.05 \) versus iliac.

b IS, injury score. IS 0, no rupture of IEL; IS<3 no rupture of EEL; IS 3 EEL ruptured.
EEL was intact (injury score < 3, n = 17 iliac and n = 12 coronary vessels) and those in which the EEL was ruptured. This demonstrated that compliance within a given vascular bed was similar for injury scores 0–2 (Table 1): that is that the internal elastic lamina offered minimal resistance to balloon expansion. However, compliance measurements were increased if the external elastic lamina was ruptured (Table 1). In addition, the compliance measurements were significantly larger where the EEL was intact in the coronary vessels than in the iliac vessels.

Rupture of the internal elastic lamina (IEL) was observed in only five of 18 iliac vessels but in 13 of 16 coronary vessels (P < 0.05), and mean injury score was 0.39 ± 0.18 in the iliac vessels and 1.63 ± 0.27 in the coronary vessels (P < 0.05). The mean intimal fracture length ratio (IELf/c) in the all coronary and iliac vessels was 0.24 ± 0.04 and 0.05 ± 0.02 respectively (P < 0.05). Exploratory analysis in only those vessels where the IEL had been broken revealed that IELf/c was still significantly greater in coronary (0.29 ± 0.04) than in iliac (0.19 ± 0.02) vessels (P < 0.05). In coronary vessels, intimal fracture length was significantly predicted by vessel compliance [(B – P)/(M − P)] (Fig. 1). Due to the low number of iliac vessels which had a fractured IEL (5/18) this relationship was not statistically significant for the iliac vessels (P = 0.45, 1 – β = 0.11). Importantly, the amount of balloon oversizing (M/P) did not predict either injury score or intimal fracture length (data not shown).

### 3.2. Angiographic acute gain and late lumen loss

Greater compliance [(B – P)/(M − P)] and rupture of elastic laminae in the coronary vessels was associated with larger post-angioplasty angiographic lumen diameter and acute gain than in the iliac vessels. However, at follow-up angiography there was greater late angiographic lumen diameter loss in the iliac group (Table 1).

3.3. Severity of injury predicts extent of neointima formation but not remodelling

For the group which were perfusion fixed and quantitative morphometry performed the preinjury vessel size (C: 2.67 ± 0.08, I: 2.64 ± 0.05 mm) and balloon oversizing (C: 1.48 ± 0.03, I: 1.51 ± 0.03) were similar between groups and representative of the whole study population. Angiographic follow-up diameter (C: 2.05 ± 0.13, I: 1.38 ± 0.12) and late loss (C: 0.98 ± 0.15, I: 1.34 ± 0.14) in the fixed group were also representative of the group as a whole. The site used in each vessel for morphometric analysis was the site of greatest injury and intima formation. In all vessels this was also the site of smallest lumen area.

The coronary vessels developed significantly more intima than the iliac arteries (Fig. 2). The amount of intima formed after balloon overstretched injury of porcine vessels was highly dependent on the depth and circumferential extent of rupture of the elastic laminae consistent with observations from previous studies [7,8]. The intimal area for the vessels was strongly correlated with severity of injury (Fig. 2), and was predicted by the following formulae:

\[
\text{Intimal area (mm}^2\text{)} = 2.53 \times \text{IELf/c} + 0.0739
\]

or

\[
\text{Intimal area (mm}^2\text{)} = 0.451 \times \text{Injury score} + 0.147
\]

Due to the widely contrasting injury scores it was not possible to determine whether vessel type had any statistically significant effect on neointimal volume independent of extent of injury. The mean (±SEM) percentage of angiographic late area loss which could be attributed to intima formation in the coronary vessels was 33 ± 8%, while in the iliac vessels it was 8 ± 3%.

Despite much lower injury score, lumen size 28 days after injury in the iliac vessels was much smaller due to reduction in total vessel area (Fig. 2). Neither histological lumen size nor total vessel area were significantly correlated with injury score in either group or in all vessels. Similarly neither angiographic late loss or the percentage of late area loss which could be attributed to inward remodelling was significantly related to injury score.

Masson’s Trichrome staining with Orcein of coronary vessels showed that extensive rupture of the elastic laminae resulted in deposition of collagen interspersed with smooth muscle cells to breach the arc of rupture. Adventitial collagen accumulation was only moderate and predominantly confined to the region of most intimal and medial damage. In contrast in the iliac vessels moderate adventitial collagen was present even in vessels without rupture of the elastic lamina (Fig. 3).
4. Discussion

In this study we have demonstrated that the pattern of healing responses after balloon injury in the coronary bed differs significantly from that seen in the iliac vasculature. These differences should be taken into account when considering the applicability of studies done in the iliac vessels to human coronary restenosis.

When compared to the iliac vessels, the coronary arteries demonstrated increased angiographic compliance, sustained more extensive injury and developed more neointima. The greater balloon expansion (compliance) observed in the coronary vessels resulted from a combination of more frequent rupture of the elastic laminae and less elastic resistance. Even in vessels where the external elastic lamina remained intact balloon expansion was significantly greater in the coronary bed. The reduced elastic resistance and higher injury scores in the coronary vessels can be at least partly attributed to differences in the underlying vessel structure, consequent to their unique origins and haemodynamic environment. More extensive injury in the coronary arteries was associated with a ten-fold increase in the size of neointima formed. While neointima formation has been previously shown to depend on the depth and extent of vessel injury [7,8] it is uncertain whether this can account for all of the observed difference between coronary and iliac responses or whether known regional variability in smooth muscle cell biology may contribute [11]. Others have recently reported greater injury and neointima formation in coronary than in carotid vessels in response to similar balloon overstretch injury [12]. Our study has extended these findings by providing direct angiographic evidence for reduced vessel elasticity in the coronary arteries and reemphasize that these unique structural characteristics have important effects on the injury which results from balloon dilatation. Perhaps more importantly, we have demonstrated important differences between coronary and peripheral beds in remodelling, which is now thought to be the main cause of restenosis after angioplasty.

The reasons underlying the differences in inward remodelling observed in the iliofemoral and coronary vessels are unclear. Previous studies have suggested that negative remodelling after injury in coronary arteries is dependent on collagen production by adventitial myofibroblasts and requires deep vessel wall injury [13–15]. Although the injury sustained in the iliac vessels was neither deep nor severe remodelling was far greater than in the coronary vessels. As restenosis and negative remodelling in injured iliac vessels has been negatively correlated with vessel wall collagen [16], it is possible that iliac artery remodelling after balloon dilatation is not a directly related to severity of injury and collagen deposition. Consistent with this possibility, inhibitors of collagen cross-linking have successfully prevented negative remodelling after injury in coronary but not iliac vessels [17,18]. The reduction in vessel wall collagen observed in restenotic iliac vessels was attributed to the pronounced induction of metalloproteinase (MMP) activity after injury in iliac vessels [19], inhibition of which significantly reduces inward remodelling [20].
Fig. 3. Morphological features of coronary and iliac vessels 28 days after injury. Perfusion fixed, paraffin sections of balloon injured coronary (left) and iliac artery (right) stained with Masson’s Trichrome with Orcein (stains smooth muscle cells red, collagen green and elastin black). Scale bars represent 1 mm. Note the EEL is thicker in the iliac vessel. After balloon overstretch in the coronary vessel, extensive vessel wall injury with rupture of IEL has resulted in significant neointima formation which consists largely of smooth muscle cells and disordered collagen. In the iliac the IEL remains intact with only a minor amount of neointima formation but a with reduced lumen size mainly due to vessel shrinkage.

Although MMP activity has been reported to be reduced in porcine and human coronary arteries after balloon injury these studies have been conducted very late in the course of restenosis and examined only the intima and media, not the adventitia where most collagen accumulates [21,22]. It is quite possible that early metalloproteinase activity may be important in inward remodelling after injury in coronary vessels.

There are some limitations of this study that should be considered. Firstly, the study was performed in normal porcine coronary and iliac vessels. The variable deformability of atherosclerotic plaque components (e.g. calcium vs lipid) may also significantly affect vessel compliance, the likelihood of tears of the elastic laminae, and response to balloon injury. Recent studies have found a similar importance for remodelling in lumen loss after balloon dilatation of artificially created atherosclerotic lesions in iliofemoral porcine vessels and naturally occurring atherosclerotic lesions in iliac vessels of cynomolagus monkey [23,24]. However there has not been a direct comparison between coronary and iliac vessels in these atherosclerotic models.

Secondly, in this study we were not able to reliably determine the absolute amount of remodelling (the change in vessel area from before balloon injury to follow-up). Two methods are commonly used to generate such data: intravascular ultrasound (IVUS) and use of a histological reference segment. Accuracy of IVUS measurements relies on consistency of vascular tone during measurement. Despite the use of intraarterial nitrates the introduction of an IVUS catheter to the vessels of our animals resulted in significant reduction in angiographic lumen size (unpublished). Reference segments, which are widely used in both histological and IVUS-based studies to define remodelling, frequently undergo remodelling after nearby vessel injury [25]. We therefore chose to control for these problems by comparing coronary and iliac arteries which were of near identical size prior to injury to detect differences in remodelling responses.

Lastly, several significant results in this study were the result of post-hoc subgroup analysis, and as such require further testing for confirmation. Alternatively, the lack of statistical power prevented critical examina-
tion of many important issues, such as the influence of smooth muscle cell heterogeneity on intima formation, and these issues may have been resolved by assessment in greater numbers.

In summary we have shown that injury inflicted by balloon dilatation and the subsequent healing responses are distinctly different between the coronary and iliac arteries justifying caution in extrapolation of data derived from injury of the porcine iliac vessels to human coronary restenosis. As the mechanisms of remodelling in primary atherosclerotic and restenotic lesions are only just beginning to be elucidated, it may be important to recognize that distinct mechanisms may be important in different vascular beds. Further investigation of the reasons underlying these differences in remodelling may shed light on some of the basic causes of this important mechanism of lumen loss in both primary atherosclerotic and restenotic lesions.

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References


