Artery wall damage and platelet uptake from so-called atraumatic arterial clamps: an experimental study

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A 'traumatic' clamps are routinely used to control arteries during reconstruction, but little is known about the arterial damage caused and the effects on platelet uptake. This experiment used sheep carotid arteries to correlate the degree of histologic damage observed with the level of indium-111 labelled platelet uptake in clamped arterial segments. Scanning electron microscopy and light microscopy enabled three degrees of injury to be recognized. In mild injuries, endothelial cell orientation was changed but local platelet uptake was little different from controls. In moderate injuries, the endothelial cells directly squeezed by the clamp were morphologically altered, superficial fissures developed which extended into the media, and local platelet uptake was usually increased. Severe injuries caused extensive endothelial cell desquamation, formation of deep cavities in the media and increased platelet uptake (mean 5.51 times that of control). Platelet uptake at the site of clamp application was not significantly different from non-clamped carotids for mild injuries. However, the increased platelet uptakes for moderate ($P = 0.007$) and severe ($P = 0.005$) injuries were statistically significant when compared with non-clamped control arterial segments. © 1997 The International Society for Cardiovascular Surgery.

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Clamps are necessary for securing arteries during reconstruction, but surprisingly few studies have investigated the acute effects of routine clamping. Partial wall injuries are sometimes recognized during operation and include intimal disruption with flap formation, dissection, occlusion and plaque fracture. Full-thickness injury may cause bleeding through the artery wall, such as occurs when a clamp is applied with excessive pressure or when the vessel is unusually fragile. Other injuries from arterial instrumentation include stripping of the intima following passage of an intra-arterial balloon, creation of arteriovenous fistulas, haemorrhage, and false aneurysm formation. All these lesions are relatively gross and are more likely when the artery is diseased.

The nature and extent of lesser damage in normal arteries from routine clamping has not been systematically studied but intimal hyperplasia and restenosis may also be related to unrecognized trauma. The etiology of intimal hyperplasia is unexplained but local thrombus deposition following trauma may contribute to thrombus forming at the site of endothelial injury may also embolize or progress to occlude a reconstructed vessel. Late strictures sometimes occur when a clamp was applied, even though no stenosis was evident in the perioperative period. In these patients presumed incomplete wall damage has healed by fibrosis with later stricture formation. An investigation of the acute effects of apparently atraumatic arterial clamping, including the extent of platelet deposition on the luminal surface and the histologic and electron microscopic changes in the wall, is reported.

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Materials and methods

Animal experiments

Anaesthesia was induced in 10 sheep (of body weight 40–50 kg) with intravenous Nembutal (15 mg/kg) followed by endotracheal intubation, oxygen, and 1.5–2.5% halothane with maintenance of systemic blood pressure at 90–100 mmHg. The neck was incised in the midline for access to the carotid arteries. A 50-ml blood sample was then withdrawn from each animal by direct puncture of the external jugular vein for platelet-labelling procedures.

Both common carotid arteries (external diameter 7–8 mm) were exposed and isolated over a length of 8–10 cm. A standard dose of intravenous sodium heparin was then administered (100 IU/kg). Five minutes later, each carotid artery was cross-clamped using two standard 45° angled DeBakey vascular clamps (Downs, England) (Figure 2). On each artery the two clamps were applied 5 cm apart for a period of 30 min. This period was chosen as it is comparable with that of clamping during many arterial reconstructions. The clamps were tested in the Department of Mechanical Engineering, University of New South Wales by a loading-cell device to measure the forces generated. Each clamp was closed three or four notches, equivalent to 0.3 to 2.5 Newtons (N), calculated by the equation:

\[ t = (0.4163 - 0.0172 \times No) \times F + (5.9957 - 1.4688 \times No) \]

where \( t \) = clamped thickness (mm), No = Notch number, and \( F \) = force applied (N).

After the clamps were released, the 30 ml of the suspension now containing autologous platelets labelled with radioactive indium oxine (\(^{111}\)In)\(^4\) was injected into the external jugular vein. One hour later the carotid arteries were excised and carefully irrigated with normal saline to remove any loose blood while retaining adherent thrombus; 1-cm segments of unclamped artery proximal and distal to the clamped segments were then resected as normal controls. A 4-ml sample venous blood was aspirated from the jugular vein for comparative analysis and the animals were killed with a lethal dose of intravenous sodium pentobarbitone.

For each carotid artery, four segments, each 1 cm long were analysed for radioactive platelet uptake. Two sections of the artery centred at each clamp application were removed, plus two segments for non-injured controls (Figure 1). Uptake was counted in a well-gamma counter (Cobra II, Hewlett Packard, USA). Each arterial specimen was incised longitudinally and prepared for light and scanning electron microscopy.

The surgical procedures were carried out at the Department for Biomedical Engineering, University of New South Wales, with the approval of the Local Animal Care and Ethics Committee. Statistical analysis was carried out using the Wilcoxon rank test with significance <5%.

Platelet preparation

The method for platelet labelling is modified from that of Desai and Thakur\(^5\) and is described in detail elsewhere\(^4\).

Briefly, the blood was centrifuged at 200 g for 20 min at room temperature. Platelet-rich plasma was separated from the red blood cells with a syringe. The platelet-poor plasma was removed by pipette and saved for later resuspension of platelets. A platelet pellet was then obtained by further centrifugation at 640 g for 10 min and the platelets resuspended in calcium-free Tyrode's solution. The labelling was done by incubation with approximately 30 \( \mu Ci \) \(^{111}\)In oxine for 1 min in the presence of a small amount of platelet-poor plasma. Labelled platelets were resuspended for re-injection.

The radioactivity of the supernatant and labelled platelets was determined in a radioisotope well-counter, following by calculation of the 'labelling efficiency' by:

\[ \% LE = AP \times 100/(AP + AS) \]

where LE = labelling efficiency, AP = activity in platelets, and AS = activity in supernatant. The mean (s.e.m.) labelling efficiency of platelets was 68.3 (9.6)% (\( n = 10 \); range 53–83%).

Scanning electron microscopy

After fixation of the arteries in 2.5% glutaraldehyde in phosphate-buffered saline, each specimen was
post-fixed in osmium tetroxide, followed by dehydration through graded (70%, 90% and 100%) ethanol and critical-point drying using CO2. The specimens were mounted and coated with 100 A of gold–palladium before scanning electron microscopy (Stereoscan S 150, Cambridge Instrument Co.).

**Light microscopy**

Samples were fixed in 10% buffered formalin. Morphological aspects of vessel alteration in different stages of damage were studied using staining of paraffin sections with haematoxylin and eosin. Sections were analysed using an Olympus BH-2 microscope and by computer imaging using the program Image Scion 1.51.

**Results**

Scanning electron microscopy and light microscopy showed three degrees of damage (Table 1).

**Scanning electron microscopy**

First-degree or mild injury affected 10 of the clamped segments (33%) and was characterized by the formation of a fold in the luminal surface (Figure 2a). Higher magnification showed changes in endothelial cell orientation without actual cell disruption. Some platelets and red blood cells were identified on the surface along the fold.

Ten (33%) clamped segments were classified as second-degree or moderate damage, in which fissures in the arterial lumen extended up to 50% of its thickness with some fissure formation (Figure 2b). Disruption of the endothelium with deposition of fibrin, aggregated red blood cells and platelets was seen around the fissure.

Third-degree or severe clamp damage (10 segments, 33%) caused much deeper and more destructive fissuring of the arterial wall with up to 100% of the medial layer involved and cavities evident in some sections. The associated zone of endothelial desquamation was extensive. Massive accumulation of platelets and red blood cells along the fissure were consistently seen in severe clamp trauma and a large mixture of fibres and thrombus formed in the bottom of the cavity (Figure 2c).

**Light microscopy**

In mild injuries, light microscopic analysis did not identify any significant endothelial injury and the internal elastic lamina was uninterrupted. Vacuolization of some smooth muscle cells in deep medial areas was evident in some specimens (Figure 3a).

In moderate injuries, vacuolization of smooth muscle cells in the media was consistently detected. The internal elastic lamina was partially interrupted in the fissure zone and the adjacent endothelial cells showed definite signs of injury within the fissure zone, without extensive desquamation of other cells (Figure 3b).

In severe injuries, the cell response was different compared with moderate injury, including complete desquamation of the endothelial cells in the zone of clamp application. Vacuolization of smooth muscle cells in the superficial and deeper part of media was apparent. Continuity of the internal elastic lamina was broken in the fissure zone, with the fissure usually extending right through the medial layer to form a cavity filled with thrombus (Figure 3c).

**Platelet uptake**

The extent to which platelets adhered to the clamped segments was compared with that in the normal segments and the ratio of their respective radioactivity uptakes calculated. This ratio was correlated with the three degrees of clamp injury recognized by the microscopy findings.

Thirteen of 30 (43.3%) arterial segments demonstrated significantly increased platelet uptake. In the remaining 56.7%, uptake ratio was not significantly increased. 111In-labelled platelet uptake was lowest in the group of clamped arteries with mild level injury and the median platelet uptake was not significantly different from that in the normal arterial segment (median ratio 0.9, non-parametric data; Figure 4). In half of the moderate injuries the platelet

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<td>Intact</td>
<td>May be disrupted</td>
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<td>Up to 50% of media</td>
<td>Up to 100% of media</td>
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Uptake was also normal but was increased in the other five clamped segments. In severe injuries, the platelet uptake was consistently increased compared with the unclamped controls (median ratio 5.51).

Discussion

Clamps specifically for securing arteries were first introduced in the early 20th century. In the 1950s and 1960s a number of different designs were developed based on the cross-membered metallic blade. These clamps exerted varying occlusion pressures and their shapes were modified according to their intended use. The clamps were described as atraumatic, but were capable of causing injury which probably related to the occlusive forces generated and the jaw-opposition geometry.

Less damage is produced when closure is sufficient to just occlude a vessel compared with when the clamp is fully closed. However, although the compression forces needed to arrest flow in a vessel distended by 300 mm Hg pressure may only amount to 50–70 g, most vascular clamps required over 1000 g of pressure to actually engage the latches.

In the present study, when the arterial clamps were closed three to four notches, the force generated on the artery wall varied from 0.3 to 2.5 N, equivalent to 300–2500 g. When the area compressed by the clamp is examined by scanning electron microscopy, any thrombus present was found to have originated from the host artery where the vascular clamps had been applied during grafting procedures.

Slayback and colleagues offered a classification of clamp-related vessel injury based on scanning electron microscopy observations of the rabbit aorta. These authors recognize four degrees of injury as follows: (i) firstly intimal distortion; (ii) intimal distortion with clot formation; (iii) intimal disruption, and (iv) complete intimal fracture. Slayback et al. used microvascular clamps which are quite dissimilar to full-sized clamps applied to large arteries. The present experiment better simulates human operating conditions and the classification that it proposes may be more appropriate. In contrast, Harvey and Gough graded the damage of the arterial wall in the following fashion: grade I, intimal damage only; grade II, minor medial damage; and grade III, major medial damage.

The present study also confirms that the severity of injury in different arteries varies, from mild intimal injuries that do not affect platelet uptake, to medial injuries in which platelet uptake is increased.

Figure 2. Scanning electron micrographs showing damage of the sheep carotid artery upon clamp application (see text for explanation). (Original magnification × 180). a, mild damage; b, moderate damage; c, severe damage.
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This is especially so in deep medial injuries in which the degree of arterial wall injury broadly correlates with the extent to which platelets adhere and aggregate locally. Mild injuries are almost certainly reversible and are characterized by little or no abnormal platelet uptake. Moderate and severe injuries, however, damage the endothelial cells to a significant extent and also cause fissuring and cavity formation in the media. The present studies do not indicate whether these changes are a prelude to chronic changes but late stricture formation at the site of previous clamp application are well recognized clinically and probably result from healing with fibrosis after medial damage.

The present authors were also concerned that, particularly with severe injuries, the uptake of thrombus locally may be considerable. In some occluded or embolizing arterial reconstructions, the source of intraluminal thrombus may be obvious, such as when intimal dissection occurs. In many patients

Figure 3 Light microscopy showing damage of the sheep carotid artery upon clamp application (explanation in text). (Original magnification × 100). a, mild damage; b, moderate damage; c, severe damage. Arrows indicate internal elastic lamina

Figure 4 Correlation between radiolabelled platelet uptake and severity of arterial wall damage (see explanation in text)
clamping, the forces generated by the clamp, and the related injuries. Meanwhile, they conclude that vascular clamps are not truly atraumatic and that the range of injuries produced range from minor and reversible to significant injuries with extensive desquamation of the intima and fracture of the full thickness of the media.

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References


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