Eur J Cardio-thorac Surg (1996) 10:684–689

ORIGINAL ARTICLE

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Loss of endothelium-mediated vascular relaxation as a response to various clamping pressures

Received: 6 February 1995
Accepted: 30 June1995

This work was supported in part by grant No. P3-0160-305 from the Ministry of Science and Technology, Ljubljana, Republic of Slovenia

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Abstract The contraction/relaxation responses of thoracic aortal rings clamped with two clamping pressures to potassium chloride (KCl), noradrenaline and carbachol were studied using a scanning electron microscope (SEM) to ascertain endothelial lacerations. Clamp A had the tip pressure $P_A = 0.60$ N/mm^2 and clamp B $P_B = 5.16$ N/mm^2. In 15 Wistar albino rats, weighing 328 ± 19 g (mean ± SD), the thoracic aorta was occluded for 15 min and then three vascular rings (2 mm wide) were excised. The proximal unclamped ring served as a control. The aorta diameter was calculated from the circumference of distal rings 1.61 ± 0.01 mm ($n = 15$, $d_{min} = 1.51$ mm, $d_{max} = 1.70$ mm). The rings were challenged with cumulative additions of KCl (10–80 mmol/l) to measure the contraction. Then cumulative relaxation on the administration of carbachol (0.01–100 μmol/l) as a response to noradrenaline precontraction (0.1 μmol/l) was determined. A significant loss ($P < 0.05$) of vascular relaxation in all clamped rings (clamped with P_A and P_B clamping pressures) was seen. No significant differences ($P > 0.05$) were observed for contraction between clamped and control rings clamped with clamp A, however the rings clamped with clamp B showed significantly reduction of contraction ($P < 0.05$). No significant differences were seen from control rings between groups A and B ($P > 0.05$), as well as from clamped rings between groups A and B ($P > 0.05$) for both the contraction and relaxation parts of the experiments. With SEM, great endothelial lacerations with complete disruption of the endothelial layer in the rings clamped with the clamp B were seen, but no disruption in rings clamped with clamp A. Therefore endothelial vascular layers are much more susceptible to pressure injuries than was previously believed. The clamped vessel wall injuries, particularly in endothelial layers, depend on the momentary peak clamping pressure (MPCP) as well as on the lower stationary clamping pressure (SCP). [Eur J Cardio-thorac Surg (1996) 10: 684–689]

Key words Endothelium-derived relaxing factor · Vascular relaxation · Vascular contraction · Clamping pressure · Endothelial injury · Scanning electron microscopy

Introduction

The short- and long-term patency of coronary artery bypass grafts depends on a wide range of vascular mechanisms that may act to guard against graft occlusion [2]. To determine the degree of various types of injuries on vessel walls [7, 8, 9], it is useful to study changes in the vascular smooth muscle tone that is modulated by the vascular endothelium [18]. The contraction/relaxation vascular reactions studied with endothelial-dependent vasodilators, which require an intact endothelium providing the endothelium-derived relaxing factor (EDRF) [18, 20], are a well established method in basic pharmacology. The endothelium may have a critical effect on the homeostasis of vessel tone, in providing protection against vasospastic events and controlling vessel reactivity [2]. Surgical handling of coronary bypass vessels can profoundly affect their biological properties. Endothelial damage occurs quite frequently, particularly in free grafts, for example saphenous vein and, if used as such, in the gastroepiploic or even internal mammary arteries (IMA) [17].

The clinical consequence of the altered vascular reactivity initiated by endothelial damage is that the bypass graft may be subject to episodes of spasm [2]. This can occur even in IMA grafts [11] despite the fact that, when perioperative damage to the graft material was examined, IMA grafts were almost free of endothelial damage [3]. The exact mechanism of the vascular responses that cause vasospasm in native coronary arteries and coronary grafts is still not clear. Furthermore, at clamping sites vessel wall damage and resulting thrombosis can easily develop [14] as a result of surgical trauma, a damaged wall reaction [9] and reaction of the vessel wall to suturing material [7], and in order to prevent thrombosis in general it is essential to traumatize tissue and organs as little as possible [8]. In order to prevent thrombosis resulting from the vessel damage at clamping sites, the clamping pressure during anastomsoing should be minimal. The minimal vessel clamping pressure should be called the clamp tip pressure that stops blood flow and prevents sliding of cut vessel ends out of the clamp tips during anastomosing. We used the measurements of the contraction/relaxation responses of vascular rings [21] clamped with different clamping pressures to various vasoactive substances to determine the exact degree of surgical clamp-produced vessel wall injuries and performed scanning electron microscope (SEM) study of the damaged vessel wall parts.

Materials and methods

Anesthesia and surgical procedure

Fifteen Wistar albino rats, weighing 328 ± 19 g (mean ± standard deviation) were intubated (through tracheostomy) and artificially ventilated, and anesthesia was obtained with an intraperitoneal bolus injection of pentobarbital sodium of 0.1 mg/kg. The animals were ventilated with 400 ml/kg per min (50 inspirations/min, each 8 ml/kg, as described by McLeod et al. [23]). The animals received human care throughout the experiment. The thoracic aorta was dissected through the 4th left intercostal space. Prior to clamping, 5000 IU/kg of the heparin was administered intravenously. After 15 min of occlusion, the animals were sacrificed and three vascular rings (2 mm wide) were excised. The clamp was not removed until the rings had been excised. The proximal unclamped ring (nearest to the aortic arch) served as a control. The diameter of the aorta was calculated from the circumference of distal rings 1.61 ± 0.01 mm ($n = 15$, $d_{min} = 1.51$ mm, $d_{max} = 1.70$ mm).

Clamping forces

The minimal (reasonable) clamping pressure (P_m) was chosen to be 3 times a vascular (arterial, venous) systolic pressure. We measured the clamping forces of two clamps commonly used in cardiovascular microsurgery and they were used as follows: the first clamp A had the tip pressure P_A approximately equal to P_m ($F_A = 2.8$ N, $w = 2$ mm, $P_A = 0.60$ N/mm^2, F = force in Newtons, w = the clamp arm width), the second clamp B had the tip pressure P_B almost 9 times greater than P_m ($F_B = 6$ N, $w = 0.5$ mm, $P_B = 5.16$ N/mm^2). The animals where aortas were clamped with clamp A were labeled as group A (nine animals), in the other six animals aortas were clamped with the clamp B (group B).

Measurements of contraction/relaxation responses

The rings were placed between two L-shaped stainless steel holders and mounted in two separate organ baths (20 ml) containing Krebs solution [22] maintained at 37 °C and continuously gassed with a mixture of 95% oxygen and 5% carbon dioxide, giving pH approximately 7.4 [1, 22]. The rings' tensions were recorded isometrically, the clamped and control rings were investigated in parallel, stretched to an initial tension of 1 g and allowed to equilibrate for 1 h [13] and then challenged with cumulative additions of potassium chloride (KCl, 10–80 mmol/l), to measure the contraction [25]. Thirty minutes after the KCl washout, the rings were precontracted by the addition of 0.1 μmol/l of noradrenaline (L-noradrenaline bitartrate), and after the maximum contractile response the cumulative relaxation on addition of carbachol (carbachol hydrochloride, 0.01–100 μmol/l) was determined as a vertical displacement from the starting position (at time = 0) [25].

Scanning electron microscopy

The maximal possible length of thoracic aorta was excised and the procedure for SEM preparation started immediately [14].

Results

The Tables 1 and 2 show the numeric results from the contraction and relaxation part of the experiment. The graphic representation is shown in Fig. 1. The Student *t*-test for independent samples showed a significant loss ($P < 0.05$) of the endothelium-mediated vascular relaxation in all clamped rings (clamped with P_A and P_B clamping pressures). No statistically significant differences ($P > 0.05$) were observed in the contraction part of the experiment

between clamped and control rings clamped with clamp A, however the rings clamped with clamp B showed statistically significant reduction of contraction in comparison with the control rings ($P<0.05$). No statistically significant differences were seen in control rings between groups A and B ($P>0.05$), as well as in clamped rings between groups A and B ($P>0.05$) for both the contraction and relaxation parts of the experiments.

Table 1 Potassium chloride-obtained contraction of clamped and control aortic rings for both groups represented in grams of tension. (*A* group A, *B* group B, *n* number of animals, *KCl* contraction of the rings on addition of KCl) Indexes KCl_{1-4} are for the following concentrations: 10, 20, 40, 80 mmol/l

Aortic rings $\bar{x} \pm$ sd	KCl_1	KCl_2	KCl_3	KCl_4	
$A_{clamped}$	0.05 ± 0.04	0.30 ± 0.15	0.59 ± 0.19	0.73 ± 0.23	$n = 6$
$A_{control}$	0.02 ± 0.02	0.22 ± 0.10	0.54 ± 0.16	0.75 ± 0.21	$n = 6$
$B_{clamped}$	0.06 ± 0.05	0.21 ± 0.06	0.37 ± 0.08	0.46 ± 0.12	$n = 9$
$B_{control}$	0.03 ± 0.02	0.18 ± 0.07	0.48 ± 0.13	0.66 ± 0.17	$n = 9$

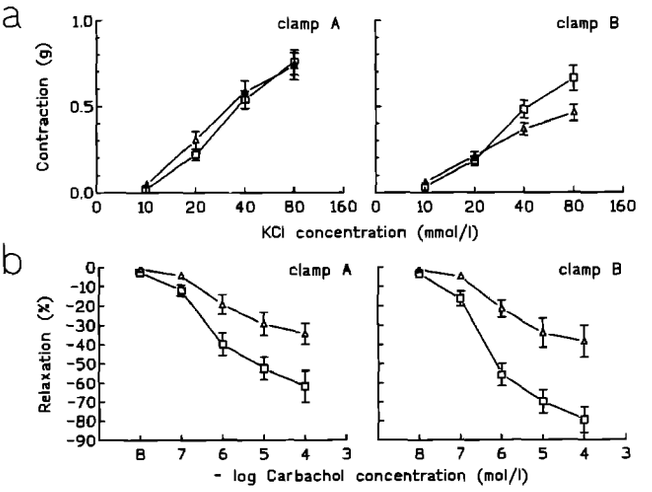

Fig. 1 Cumulative concentration-response curves for rings from rat thoracic aortas for contractile (**a**) responses to KCl (control (□) and tightened (△) rings were clamped with clamp A and B, respectively), and for relaxant (**b**) responses to carbachol (rings were precontracted by 0.1 μmol/l of noradrenaline). Each value is the mean ± SEM ($n=6$ or 9). Contraction is expressed as an increase in grams of tension, and relaxation in percentages

Fig. 2 Measured force (*F*, in Newtons) on the clamp arm while closing the rat thoracic aorta with external diameter d = 1.68 mm, wall thickness 0.10 mm and maximal joint area 4.65 mm^2. The x axis describes two parameters: a) time (in seconds), which is continuous and b) the distance between the lower and upper clamp arms (*z*) described as the displacement of clamp arms from the starting position at non-clamped (native) aorta. These are discrete points at x axis marked with the corresponding numbers. The time is in seconds, where 15 s are the distance on x axis between the two clamp arm displacements. The values for z are in mm

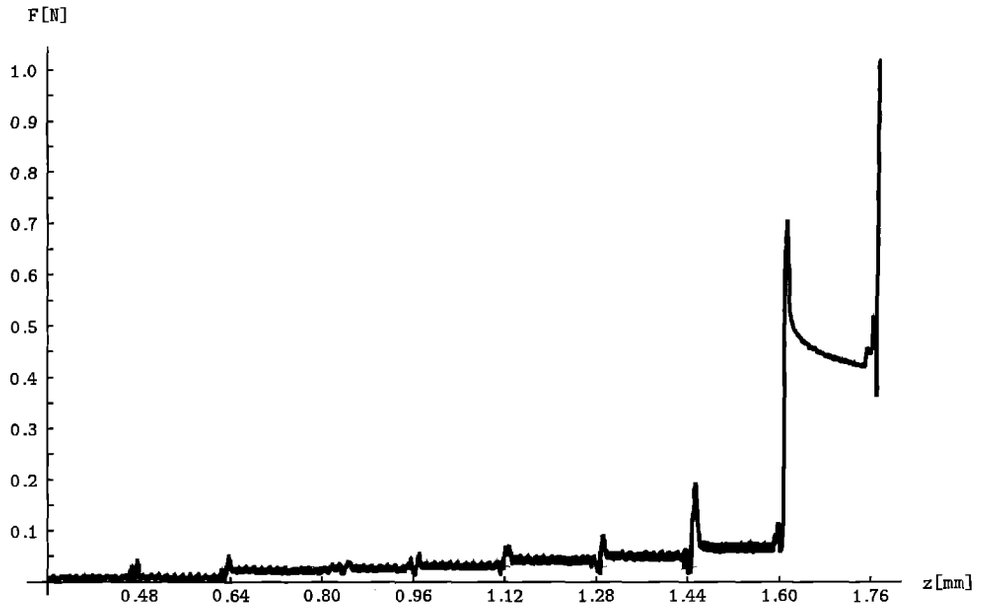

Table 2 Carbachol-obtained relaxation of clamped and control aortic rings for both groups represented in percents of maximal noradrenaline contraction. (*A* group A, *B* group B, *n* number of animals, *K* relaxation of the rings on addition of carbachol) Indexes K_{1-5} are for the following concentrations: 0.01, 0.1, 1.0, 10.0, 100.0 μmol/l

Aortic rings $\bar{x} \pm$ sd	K_1	K_2	K_3	K_4	K_5	
$A_{clamped}$	0.84 ± 1.43	4.36 ± 5.52	19.37 ± 15.66	29.42 ± 17.77	34.44 ± 16.77	$n = 6$
$A_{control}$	2.58 ± 2.39	11.97 ± 8.53	39.83 ± 17.89	52.29 ± 17.77	61.78 ± 24.79	$n = 6$
$B_{clamped}$	1.17 ± 1.81	4.90 ± 2.40	21.70 ± 11.43	34.20 ± 18.13	38.52 ± 20.42	$n = 9$
$B_{control}$	3.45 ± 2.67	16.45 ± 9.49	55.82 ± 14.13	69.98 ± 15.48	79.59 ± 16.38	$n = 9$

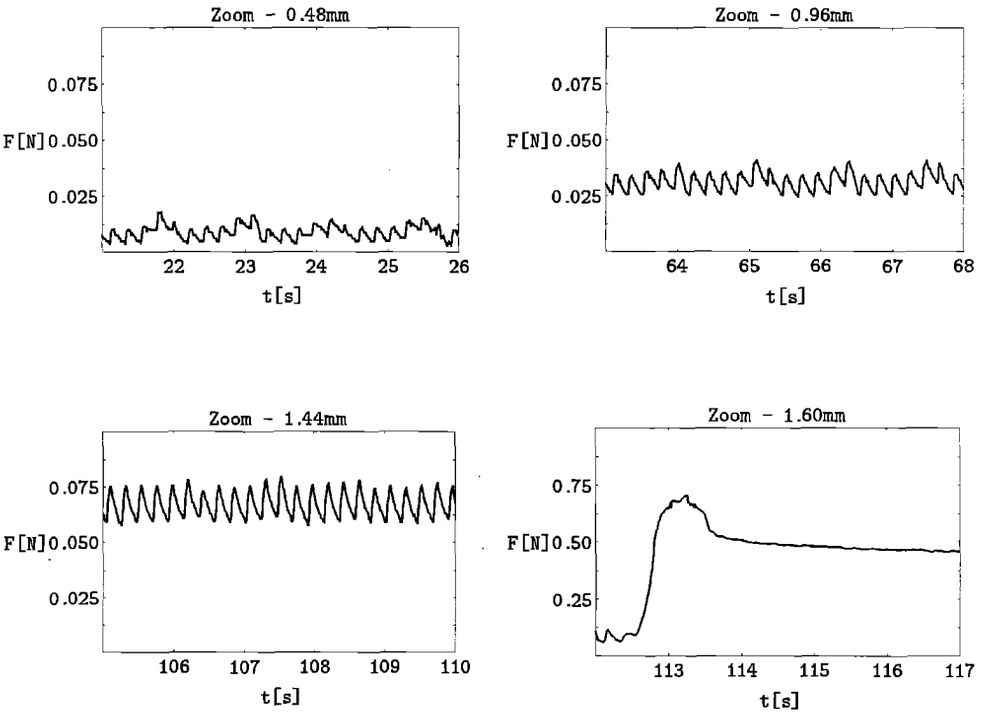

Fig. 3 Zoom – 0.48 mm. The zoomed section from Fig. 2 where the starting difference between the clamp arms (1.68 mm) was lessened for 0.48 mm and the force F was measured (N=Newtons, t=real time in seconds). Zoom – 0.96 mm. The zoomed section from Fig. 2 where the starting difference between the clamp arms was lessened for 0.96 mm. Zoom – 1.44 mm. The zoomed section from Fig. 2 where the starting difference between the clamp arms was lessened for 1.44 mm. This is the point on the verge of vessel closing. Zoom – 1.60 mm. The zoomed section from Fig. 2 where the vessel is closed

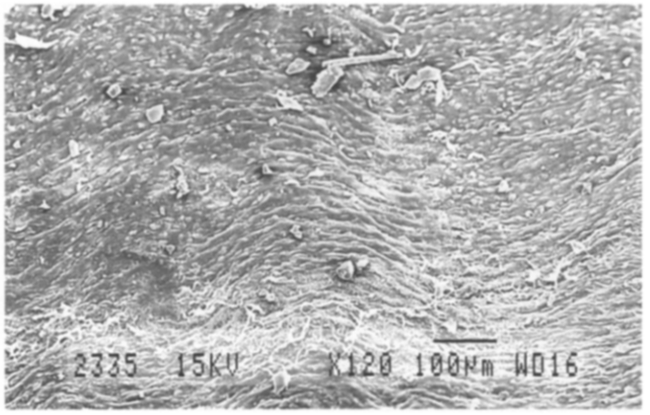

Fig. 4 Rat thoracic ring clamped with the surgical clamp producing clamping pressure 3 times greater than the corresponding systolic pressure. Scanning electron microscope: original magnification ×84, scale bar = 100 µm

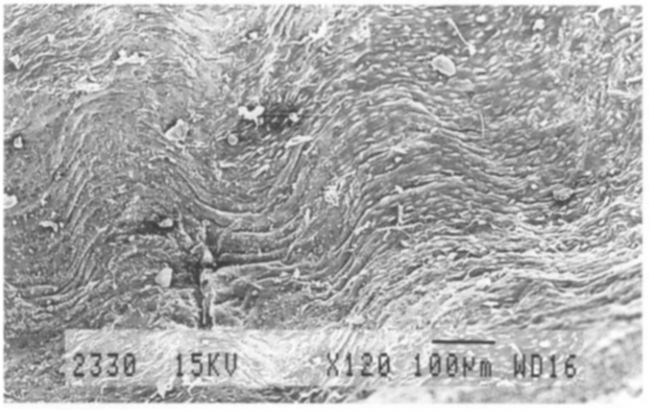

Fig. 5 Rat thoracic aortic ring clamped with the surgical clamp producing clamping pressure 9 times greater than the corresponding systolic pressure. The area clamped shows the notch, endothelial layer is disrupted. Scanning electron microscope: original magnification ×83, scale bar = 100 µm

The measured clamping force function on a rat thoracic aorta is given in Fig. 2. The distance between clamp arms was lessened in steps of half the screw revolution (0.16 mm) every 15 s, consequently the measured function was recorded in a stepwise fashion. On transitions between two neighboring steps, magnified forces can be noticed because of the manual adjustment of the screw position. The function has two parts. Before vessel closing, the clamping forces increased in proportion to the variation of the distance between clamp arms, because the joint area (A) between the clamp arms and the vessel wall was also proportional to this distance. The clamping force was modulated in this part with the force induced by the variable blood pressure. After the closing point, the average slope of the force function increased rapidly. Any attempt to decrease the distance between the clamp arms significantly could clip through the clamped vessel because the clamping forces were increasing at this time according to the strength of the clamp and to vessel characteristics. Details from Fig. 2 are shown on Fig. 3 Zoom – 0.48 mm, Zoom

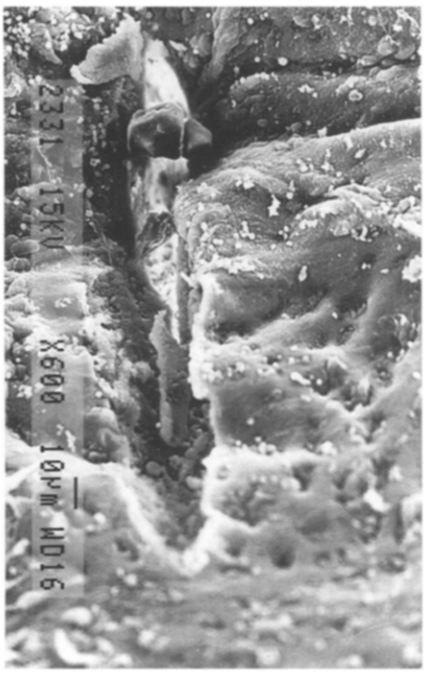

Fig. 6 Magnified damaged area from Fig. 5. Scanning electron microscope: original magnification ×426, scale bar = 10 µm

– 0.96 mm, Zoom – 1.44 mm, and Zoom – 1.60 mm. In Fig. 3 Zoom – 0.48 mm and Zoom – 0.96 mm, distances between the clamp arms are lessened for 0.48 mm and 0.96 mm, respectively. In addition to the regular pulse, a low frequency modulation, originated from breathing, can be noticed. In Zoom – 1.44 mm, on the verge of vessel closing, the systolic and diastolic pressure can be read. In Zoom – 1.60 mm, the vessel is closed. At first, the clamping force increases rapidly to the momentary peak clamping force (momentary peak clamping pressure, MPCP), but then, as a consequence of a time dependent vessel wall thinning, the clamping force decreases and stabilizes on a stationary value (stationary clamping pressure, SCP) that is approximately six times greater than the force generated by the systolic pressure.

Figure 4 shows undamaged endothelial layers from an aortic ring clamped with the clamp A. The endothelial cells are arranged horizontally, without disruption. The endothelial layer from a specimen clamped with the clamp B (Fig. 5) shows the cells disrupted, the notch goes deep towards the muscular layers (Fig. 6).

Discussion

Vascular contraction depends on relatively intact muscular layers [16, 19]. Studies of the damaging effects on transluminal angioplasty have shown the decrease of arterial contractility [26]. Some authors [4] report complete loss of immediate iliac artery contraction on the addition of KCl after balloon angioplasty. The maximal contractile response to KCl remained decreased even after 2–4 weeks [16]. These data suggest damage of smooth muscle cells and greater functional loss, as could be seen from the morphological analyses. Microscopic calcifications in the vessel walls at sites where no surgical procedure had been performed could be seen [9]. In our study we observed the statistically significant decrease of contraction in aortic rings clamped with clamp B in comparison with the control rings. However this reduction was only seen at the KCl concentration 80 mmol/l. The rings clamped with clamp A did not show such a reduction of contraction.

The endothelium-dependent relaxation is directly related to the intact endothelium [6]. The clinical consequence of altered vascular reactivity initiated by endothelial damage in surgical patients is that the bypass grafts may be subject to episodes of spasm [15]. All these studies are important for judging the possibility of the appearance of angiospasms as a result of clamping damage. All our rings relaxed, however the comparison of relaxation between control and clamped rings (for A and B clamps) showed statistically significant loss of relaxation in all clamped rings compared with control rings. This was seen also in the rings clamped with approximately 3 times rat systolic pressure, suggesting this clamping pressure (although very low) is high enough to damage the endothelial layers to such an extent that the endothelium-mediated relaxation is decreased. The clamps were not removed from the vessel until the animal was sacrificed. Reperfusion in vivo per se could add to the endothelial injury, so we could speculate that the damage in real surgical conditions is even greater than in our experiment. We believe exact studies have to be undertaken in the future to highlight this problem.

Experiments [5] performed with coronary arteries show unexpected tolerance of endothelium-mediated relaxation to global myocardial ischemia. The true reason for the coronary artery dysfunction was probably the endothelial injuries, since the disturbed production and action of endothelium-derived relaxing factor (EDRF) is one of the causes of vascular spasm [24]. We demonstrated that the reduction of smooth muscle contraction, and the endothelium-dependent relaxation were not related, which is similar to the results of intraluminal papaverine treatment in the human IMA that causes a reduction of the smooth muscle contraction, but does not impair endothelium-dependent relaxation [12]. However the SEM experiments on human IMAs [14] lack information on the exact clamping pressures (MPCP and SCP), which is understandable since such measurements are only possible in experimental conditions at present.

The clamping pressure of clamp A did not produce disruption of the endothelial layer to such an extent as the experiment with clamp B showed. However studies should

be undertaken to quantify exactly the area of endothelial damage seen on SEM after different clamping pressures, perhaps with the computerized image analysis system [10]. We believe the complete disruption is due to MPCP rather than SCP, and that the endothelial detachments seen in the human IMA study [14] are the result of SCP, surgical handling, or both. All these could suggest the use of clamps with minimal clamping pressures for each type and size of vessels to diminish the endothelial lacerations, which could possibly lead to thrombosis, and the use of non-endothelium-dependent vasodilators to prevent postoperative arterial spasms [11].

We conclude that even the minimal clamping pressure (here defined as 3 times systolic pressure) is high enough to damage the vascular endothelial layer to such an extent that a significant loss of endothelium-dependent vascular relaxation is seen, conditioned with the normal production of EDRF, and that vessel injuries, particularly in endothelial layers, depend on the MPCP as well as on the lower SCP. For long-term patency of bypass grafts it is therefore necessary to keep the vascular segments functionally and morphologically as intact as possible, since morphological and/or functional regeneration is an important factor in the appearance of restenosis [6].

Acknowledgement We are grateful to Ms. Marija Vucinic from the Research and Development Department, LEK Pharmaceuticals, for her excellent technical assistance.

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