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Long-term reduction of intimal hyperplasia by the selective alpha-1 adrenergic antagonist doxazosin

Studies have shown that α_1-adrenergic blockade reduces intimal hyperplasia in the rabbit aorta. In this study a selective α_1-adrenergic antagonist, doxazosin, has been used to examine whether this effect is persistent and dose dependent. Forty-eight New Zealand White rabbits underwent endothelial denudation of the abdominal aorta using a Fogarty balloon catheter. Test rabbits were given low-dose (2 mg) or high-dose (8 mg) doxazosin daily and all animals killed at either 1 or 12 weeks after the procedure. The aortas were harvested after fixation in situ *with 4 per cent glutaraldehyde and neointimal hyperplasia was measured, using an* x–y *digitizer, as the percentage reduction in luminal cross-sectional area. At 1 week after surgery, rabbits receiving the low dose had a median area reduction of 7·7 per cent and those receiving the high dose a reduction of 8·2 per cent; both had significantly less intimal hyperplasia than control rabbits, which had a median area reduction of 14·8 per cent ($P < 0·01$). However, at 12 weeks, when compared with the 32·6 per cent reduction in the control group, only those rabbits receiving high-dose doxazosin had significantly less intimal hyperplasia, with a reduction of 5·5 per cent ($P < 0·001$). It is concluded that selective α_1-adrenergic blockade significantly reduces neointimal hyperplasia, that this effect is dose dependent, and that it persists for at least 3 months.*

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During most vascular procedures a degree of endothelial damage is inevitable. This is followed by smooth muscle cell proliferation, which can lead to varying degrees of restenosis and even occlusion. Pharmacological attempts to control this hyperplastic response have shown promising results in animal models, but a suitable agent has not yet been identified in humans. Recent animal studies have demonstrated that endothelial denudation and the subsequent intimal hyperplasia is accompanied by a selective increase in sensitivity to noradrenaline[1–4]. This is associated with an increased affinity for noradrenaline in the α_1-adrenergic receptor of vascular smooth muscle cells and also an associated increase in turnover in the intracellular phosphatidylinositol cycle[5].

Many growth factors including platelet-derived growth factor (PDGF), fibroblast growth factor, epidermal growth factor, insulin-like growth factor, interleukin 1 and transforming growth factor are involved in the development of intimal hyperplasia[6]. Interestingly, some of these also act through the phosphatidylinositol cycle, byproducts of which lead to an increase in intracellular calcium concentration and stimulate protein kinase C to promote a series of phosphorylations, leading to cell mitosis[7]. This common pathway for α_1-antagonists and growth factors may explain previous studies showing a 40 per cent reduction in rabbit intimal hyperplasia with prazosin[8]. However, the duration of this effect is not established; nor is it known whether it is dose related.

The aim of the present study was to test whether a more selective α_1-antagonist (doxazosin mesylate) might have an even greater effect than prazosin, to investigate the duration of this effect and assess whether it is dose dependent.

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Materials and methods

Forty-eight female New Zealand White rabbits (weight 2·5–3·5 kg) underwent endothelial denudation of the abdominal aorta using a Fogarty balloon catheter. Sixteen animals were treated with doxazosin (2 mg) and another 16 with doxazosin (8 mg) from the day of surgery until they were killed; a further 16 rabbits acted as controls. The extent of intimal hyperplasia was compared at 1 and 12 weeks after endothelial denudation. Doxazosin was administered orally in a once-daily dosage; previous studies have shown that up to 40 mg per day can be given safely[9].

Endothelial denudation

The technique for endothelial denudation was a modification of that of Baumgartner and Studer[10] and has been previously described[2]. Anaesthesia was induced with ketamine (25 mg kg^{-1}) and midazolam (2 mg kg^{-1}) and maintained with nitrous oxide (2 l min^{-1}) and oxygen (2 l min^{-1}). Through a groin incision the superficial femoral artery was mobilized and a transverse arteriotomy made. A 4-Fr Fogarty catheter was introduced and advanced proximally for 16 cm, inflated with air and then withdrawn. This was repeated four times. The superficial femoral artery was ligated and the skin sutured.

Protocol

All rabbits were caged individually, given water *ad libitum* and fed normal chow. Of the 48 animals, 16 were used as controls, with half being killed at 1 week and the other half at 12 weeks. The remaining 32 rabbits were given doxazosin at either the lower dose (2 mg; $n = 16$) or the higher dose (8 mg; $n = 16$). These two groups were then divided equally into those killed at 1 week and at 12 weeks. There was thus a total of six groups:

1. Control group killed at 1 week ($n = 8$).
2. Control group killed at 12 weeks ($n = 8$).
3. 'Low-dose' group killed at 1 week ($n = 8$).
4. 'Low-dose' group killed at 12 weeks ($n = 8$).
5. 'High-dose' group killed at 1 week ($n = 8$).
6. 'High-dose' group killed at 12 weeks ($n = 8$).

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Tissue harvesting

Under general anaesthesia the internal jugular vein was cannulated and a 4 per cent glutaraldehyde solution infused at arterial pressure to fix the vasculature *in situ*. A total of 200 ml was infused over a period of 20 min and the aorta then exposed and clamped at the level of the renal arteries and at the aortic bifurcation. This segment of abdominal aorta was then excised and immediately placed in 3·7 per cent formaldehyde. This technique is a modification of that described by Clowes *et al.*[11].

Histology and morphometry

Tissue blocks of cross-sections of aorta were cut at a fixed point 2 cm distal to the renal arteries. The blocks were dehydrated with graded alcohol and embedded in paraffin wax. A total of 48 sections (one from each rabbit aorta) 4-μm thick were then cut in cross section, mounted on glass slides, and stained with haematoxylin and eosin.

Sections were then projected at a standard magnification using the Image Analysis System (Cambridge Electronic Design, Cambridge, UK). Computer-generated image analysis was used to measure the area of the neointima. All measurements were made by two observers in 'blind' fashion. The results are expressed as the percentage reduction of luminal cross-sectional area caused by intimal hyperplasia.

Statistical analysis

The reduction of luminal cross-sectional area caused by intimal hyperplasia for each rabbit was calculated and the median (range) percentage luminal reduction for each group derived. The Mann–Whitney *U* test was used for statistical evaluation of the differences in the development of intimal hyperplasia between the control and treated groups. Results are considered significant at $P < 0·05$.

Table 1 *Luminal reduction*

	Control	Doxazosin	
		Low dose	High dose
1 week	14·8 (6·6–40·0)	7·7 (2·7–10·1)*	8·2 (0·9–11·5)†
12 weeks	32·6 (18·1–44·8)	25·7 (5·5–50·3)‡	5·5 (2·7–16·2)†

Values are median (range) percentages. *$P < 0·01$; †$P < 0·001$; ‡P not significant (*versus* control group, Mann–Whitney *U* test)

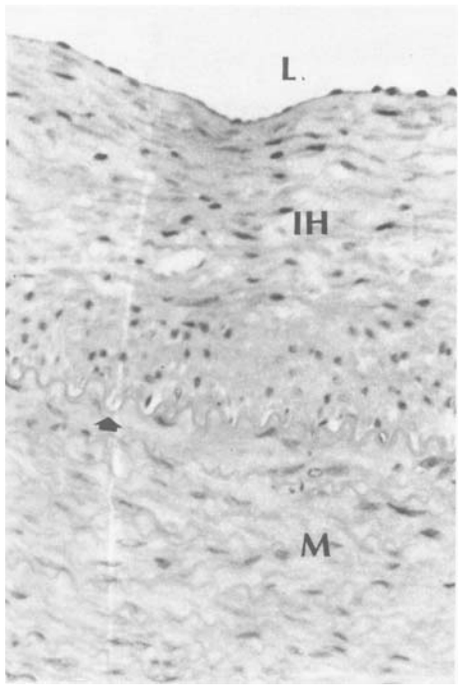

Figure 1 *Photomicrograph of a transverse section of control rabbit aorta 12 weeks after endothelial denudation showing many layers of smooth muscle cells in the neointima. L, lumen; IH, intimal hyperplasia; M, media. The arrow points to the internal elastic lamina. (Haematoxylin and eosin stain, original magnification × 90)*

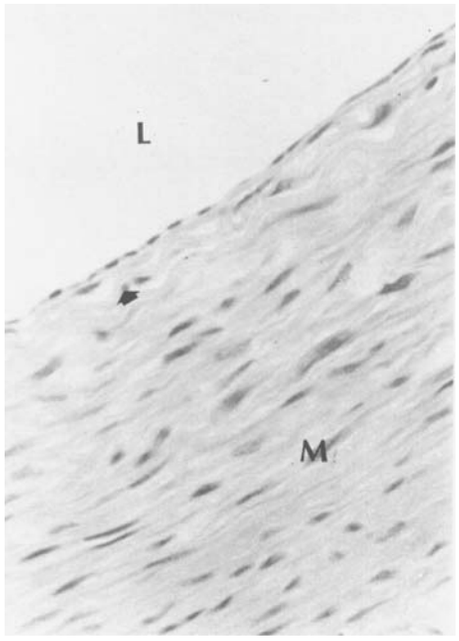

Figure 2 *Photomicrograph of a transverse section from a doxazosin-treated rabbit (high-dose group) 12 weeks after endothelial denudation showing minimal intimal hyperplasia. L, lumen; M, media. The arrow points to the internal elastic lamina. (Haematoxylin and eosin stain, original magnification × 150)*

Results

All rabbits survived the procedure and doxazosin was well tolerated. The results presented are therefore from all 48 rabbits. As in previous studies using this model, intimal hyperplasia was often asymmetrical. Rabbits killed at 1 week already showed early development of intimal hyperplasia with smooth muscle cell proliferation in the neointima; at 12 weeks the degree of hyperplasia had advanced significantly (*Table 1*). At this stage the neointima was lined by typical endothelial cells (*Figure 1*).

Both high- and low-dose doxazosin reduced intimal hyperplasia at 1 week but this did not persist in both groups at 12 weeks (*Table 1*). The median (range) reduction in luminal area in the low-dose group was 7·7 (2·7–10·1) per cent and in the control group 14·8 (6·6–40·0) per cent after 1 week ($P < 0·01$). However, the difference between these groups after 12 weeks was not statistically significant.

Intimal hyperplasia was also reduced with the higher dose of doxazosin. The median (range) reduction in luminal area was 8·2 (0·9–11·5) per cent at 1 week, statistically significant compared with 14·8 (6·6–40·0) per cent in the control group ($P < 0·001$). After 12 weeks, rabbits receiving the higher dose had developed significantly less intimal hyperplasia (*Figure 2*) than both control animals and those receiving the lower dose. The respective median (range) reductions were 5·5 (2·7–16·2), 32·6 (18·1–44·8) and 25·7 (5·5–50·3) per cent.

Discussion

Luminal narrowing from excessive intimal hyperplasia was first identified by Imparato *et al.* as a cause of late vein bypass graft failure[12]. Intimal hyperplasia has also been reported at endarterectomy sites[13], in prosthetic grafts[12,14], as recurrent stenosis after carotid endarterectomy[15] and in veins used in aortocoronary bypass grafting[16]. Less invasive procedures such as balloon dilatation angioplasty and mechanical atherectomy are also affected by intimal hyperplasia[17,18], the clinical significance of which may be even greater as it may also predispose to the development of atherosclerosis[19,20].

The rabbit is a suitable model for investigating intimal hyperplasia because endothelial denudation results in predict-

able smooth muscle cell proliferation[21]. Similar changes have recently been seen in human arteries after passage of an embolectomy balloon catheter (unpublished observations).

Several pharmacological agents have been used to modify the development of intimal hyperplasia. Aspirin and dipyridamole are frequently employed antiplatelet drugs. The results from many of the studies using such drugs have, however, been equivocal or contradictory and appear to be species dependent. A significant reduction in intimal hyperplasia has been shown in peripheral bypass in primates[22] and in canine aortocoronary bypass grafts[23]. However, antiplatelet therapy in rabbit aorta or canine peripheral bypasses has either no benefit[24] or actually increases the amount of hyperplasia[25]. The results from clinical trials are even more contradictory[26,27]. Calcium channel antagonists, anticoagulants and steroids have also been investigated in experimental and clinical studies, but without much success. The angiotensin-converting enzyme (ACE) inhibitors have caused significant reduction in hyperplasia after balloon denudation of rabbit aorta[28], a finding further supported by similar results in rabbit vein bypass grafts[29], but the reduction was less dramatic in the latter. Clinical studies with ACE inhibitors are awaited. Anticoagulants have stood the test of time and there is experimental evidence to suggest that heparin also reduces the development of hyperplasia[30], although controlled clinical studies are lacking.

The results of the present study confirm the hypothesis that α_1-adrenergic blockade reduces the development of intimal hyperplasia and that this effect is dose dependent. The higher dose reduced the development of intimal hyperplasia significantly at 1 week and this persisted for up to 3 months. On the other hand, it is interesting to note that the lower dose was effective in reducing intimal hyperplasia in the short term but ineffective at 12 weeks. The reason for this is not obvious but may be that the initial mitogenic stimuli are derived from extrinsic sources (fibroblasts, platelets etc.) but after a few days smooth muscle cells are known to produce intrinsic growth factors capable of perpetuating this proliferation[31]. Therefore, as the intensity of the mitogenic stimulus increases, perhaps higher levels of the drug are required.

There is increasing evidence to suggest a role for α-adrenergic blockade when endothelial denudation is anticipated. First, there is increased sensitivity to noradrenaline in denuded aorta[1]; second, catecholamines can stimulate *in vitro* smooth muscle cell proliferation and this is mediated through α-adrenergic receptors[32–34]; third, catecholamines can stimulate PDGF production in rat aorta[35]; and finally, α_1-adrenergic blockade can reduce the development of intimal hyperplasia by as much as 80 per cent. The developing association between adrenergic antagonists and smooth muscle cell mitogens is likely to be due to their common metabolic pathway, the phosphatidylinositol cycle.

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Surgical workshop

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Simplified method of subcuticular skin closure

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The use of subcuticular sutures is associated with a low rate of wound complications[1–3]. Many surgeons retain such a suture by either taping the cut ends to the skin or knotting the suture over the skin for later trimming or removal. Others prefer to bury an absorbable suture completely in the subcutaneous tissues, with a knot used at either end of the wound.

For those concerned about wound complications, the first approach has the attraction that the suture can be withdrawn if a problem occurs. A buried knotted suture precludes easy removal even if the wound is opened.

A completely buried skin closure does, however, remove a potential portal of entry for infection and the wound will not require further attention, such as trimming of suture ends. A buried but knotless method of skin closure is presented.

Surgical technique

Undyed polydioxanone (PDS; Ethicon, Edinburgh, UK) is used with a straight cutting needle. Closure is begun with the suture passed as a generous bite through the subcutaneous tissues. Using the exiting suture as a retractor, the needle is then passed back through the exit point for a second bite of the subcutaneous tissues, before the needle is reversed to exit at the apex of the wound. The wound is then closed in a normal subcuticular fashion. At the opposite end of the incision the process is repeated (*Figures 1* and *2*).

Before the suture is cut, the wound is cleaned and dried, and suture tension adjusted. Only then is the PDS cut flush with the skin, the ends being allowed to retract and the dressing applied. Occasionally the lower end of a midline abdominal incision will gape a little; this is usually apparent before the suture is cut and a Steri-Strip (Ethicon) can be used to buttress the wound.

Results

The use of knots with subcuticular skin closure is associated with ulceration through the skin in up to 17 per cent of cases[1]. The suture described requires no further attention. If infection occurs, the incision can be opened to allow the collection to drain and, if necessary, the suture material can then simply be removed in part or completely as no knot exists. In 247 wound closures, six patients have developed late gaping, all satisfactorily controlled with skin tapes. Infections have been managed as described without undue complication.

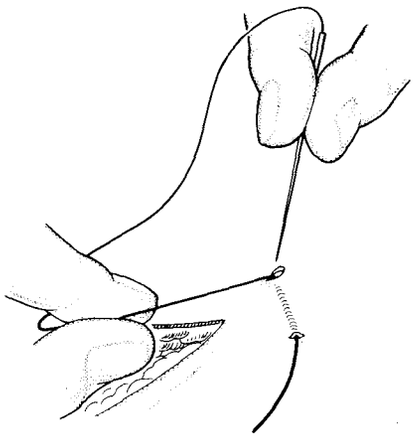

Figure 1 *The suture is used as a retractor to allow the needle to pass back through the exit point*

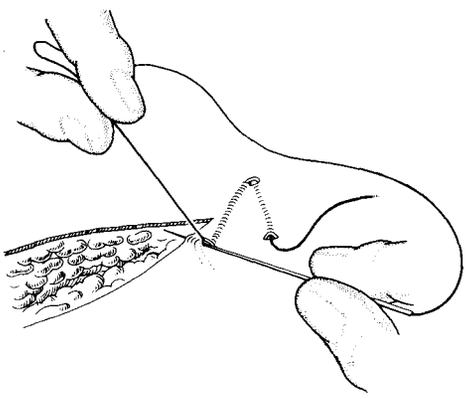

Figure 2 *The process is reversed, allowing the needle to exit at the apex of the wound*

The method is a little quicker to perform than other approaches and requires less subsequent attention.

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