Title page

Endothelin-1, endothelin receptor antagonists and vein graft occlusion in coronary artery bypass surgery: twenty years on and still no journey from bench to bedside.

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Abstract

The saphenous vein is the most commonly used bypass graft in patients with coronary artery disease. During routine coronary artery bypass grafting the vascular damage inflicted on the vein is likely to stimulate the release of endothelin-1, a potent endothelium-derived vasoconstrictor that also possesses cell proliferation and inflammatory properties, conditions associated with vein graft failure. In both *in vitro* and *in vivo* studies, endothelin receptor antagonists reduce neointimal thickening. The mechanisms underlying these observations are multi-factorial and include an effect on cell proliferation and cell/tissue damage. Much of the data supporting the beneficial action of endothelin-1 receptor antagonism at reducing intimal thickening and occlusion in experimental vein grafts was published over 20 years ago. The theme of the recent ET-16 conference in Kobe was "Visiting Old and Learning New". This short review article provides an overview of studies showing the potential of endothelin receptor antagonists to offer an adjuvant therapeutic approach for reducing saphenous vein graft failure and poses the question why this important area of research has not been translated from bench to bedside given the potential benefit for CABG patients?

Key words: endothelin-1, endothelin A receptor, endothelin B receptor, endothelin receptor antagonists, coronary artery disease, saphenous vein, bypass graft, neointima, patency

Introduction

Coronary heart disease (CHD) is the leading cause of death worldwide with an estimated 3.8 million men and 3.4 million women dying each year from this condition (Mackay et al. 2004). Coronary artery bypass surgery (CABG) is performed in over 1 million patients a year in order to restore myocardial blood supply. The saphenous vein (SV) is the most commonly used conduit for CABG since its introduction by Favaloro 50 year ago (Favaloro 1969). The SV is most frequently used since it possesses a number of practical advantages: it is expendable, since lower limb venous drainage can rely solely on the deep venous system; its superficial position renders it easily accessible, facilitating its exposure at harvest (Tsui and Dashwood 2002). However, approximately 50% of SV grafts occlude within 10 years with patients requiring reoperation to restore myocardial blood supply (Mehta et al 1997). The harvesting technique, as described by Favaloro, states "Care must be taken to dissect only the vein, avoiding as much as possible the adventitia that surrounds it" (Favaloro 1969). This has become the conventional method of preparing the SV and is used in most cardiac centres worldwide when performing CABG. However, when harvesting the SV in such a way, considerable vascular damage is inflicted, damage that affects graft quality and performance. A high proportion of SVs go into spasm during CABG due to surgical trauma and direct handling of the vein by surgical instruments. This is overcome using 'manual', intraluminal, saline distension at pressures approaching 700 mm Hg on SV explants in patients undergoing CABG (Bonchek 1980). In addition, surgical trauma at harvesting causes damage to the SV intimal endothelium, vascular smooth muscle cells, vasa vasorum and vascular nerves (Souza 1996, Ahmed et al. 2004, Vasilakis et al. 2004, Loesch et al. 2006, Dashwood and Loesch 2009, Loesch and Dashwood 2009, 2018, Dreifaldt et al. 2011, Verma et al. 2014). This combination of distension and surgical trauma may not only have immediate effects on graft patency, for example those associated with vasospasm, but also have medium- and long-term effects that lead to thrombotic occlusion, neointimal hyperplasia or accelerated atherosclerosis.

A number of studies into the potential role of ET-1 in vein graft occlusion have been performed, *in vitro*, on SV segments obtained from patients undergoing CABG as well as in *in vivo* experimental animal bypass models. Selective ETA/ETB and dual receptor antagonists reduce neointimal hyperplasia and may provide an adjuvant therapeutic approach for reducing the degree of restenosis and SV graft failure in patients undergoing CABG. To our knowledge no studies to date have explored the potential effect on clinical outcome of endothelin receptor antagonists in CABG patients.

Much of the data presented in this review was published over 20 years ago and, although of potential benefit to patients undergoing CABG, has not so far translated from bench to bedside. With the recent repurposing of certain ET receptor antagonists, perhaps now is the time to follow up the promising data obtained in these studies in order to provide an additional strategy aimed at improving SV graft patency and reducing the current requirement for reoperation on patients whose grafts have failed.

Background

ET-1 was first isolated from cultured pig endothelial cells and shown to possess a potent vasoconstrictor action in the classic study published in Nature by Yanagisawa et al. (1988).

In the following 30 years many other actions of ET-1 have been described in a variety of organs/tissues and implicated in many pathological states. For example, there is a plethora of information where selective and dual receptor antagonists have been suggested to have therapeutic potential in various conditions. Such conditions include hypertension, renal disease, occlusive vascular disease, coronary artery disease, restenosis, atherosclerosis and transplant-associated arteriosclerosis, pulmonary arterial hypertension, congestive heart failure and left ventricular dysfunction (see Lüscher and Barton 2000). In addition, the ET receptor antagonists, macicentan and bosentan, have been used to treat digital ulcers (Combalia et al 2018; Gonçalves and Santos 2019). Also, the therapeutic potential of ET receptor antagonists have been studied in patients with prostate cancer and the selective ETA receptor antagonist, zibotentan, in colorectal and other cancers (Haque et al 2014, Bagnato et al 2011). However, despite early promising data from clinical trials certain endothelin receptor antagonists were not well tolerated, exhibiting a variety of side effects, causing a number of compounds to be withdrawn. Such side effects include increased heart rate, facial flush and/or facial edema (possibly due to cerebral vasodilatation), headache, nausea, vomiting and constipation. ET antagonists may also interfere with anticoagulants, angiotensin-converting enzyme (ACE) inhibitors and cause hepatotoxicity (Lüscher and Barton, 2000; Dashwood and Tsui 2002; Davenport et al 2016; Barton and Yanagisawa 2019, Dhaun and Webb 2019).

In vitro **studies**

One of the first studies into the localization and vasoconstrictor action of ET-1 employed a combination of *in vitro* receptor autoradiography and organ bath studies (Dashwood, Turner, Jacobs 1989). Here a dose-dependent vasoconstriction was shown via ET-1 receptors located on vascular smooth muscle of isolated rabbit blood vessels. Further studies, on isolated human epicardial coronary arteries, showed similar results where ET-1 produced a long lasting, dose-dependent, increase of tension via specific receptors on the vessel wall (Chester et al. 1989) with similar data published a decade later for the human SV (Maguire and Davenport 1999). The action of ET-1 on ETA (constrictor) and ETB receptors (dilator) is well established (Lüscher and Barton, 2000, Barton and Yanagisawa 2019). For example, the binding of ET-1 to ETA receptors (on vascular smooth muscle cells) leads to an accumulation of intracellular calcium (Goto et al 1989; Yang et al. 1990) which, in turn, causes a long-lasting vasoconstriction. In contrast, the activation of endothelial ETB receptors

(on vascular endothelium) stimulates the release of NO, promoting vasorelaxation (Lüscher and Barton 2000). Since the vasomotor effect of ETA over ETB receptor activation generally predominates it therefore seems logical to target this receptor in SV grafts, as discussed later. In general, the use of ETB receptor antagonists may be inadvisable in SV grafts used in CABG since by, blocking the dilator action of ET-1 on this receptor, its constrictor effect on ETA receptors would prevail, a situation possibly accounting for the spasm that occurs at harvesting as well as other aspects underlying short-term graft patency. The diverse properties and distribution of ETA and ETB receptors on SV grafts has yet to be fully explored in order to establish their clinical potential in CABG patients.

Apart from its well-established vasoconstrictor action (Yanagisawa et al. 1988; Lüscher and Barton 2000; Barton and Yanagisawa 2019) it was not long before ET-1 was demonstrated to possess potent cell proliferation effects, in particular on vascular smooth muscle cells (Suzuki et al.1991; Scott-Burden et al. 1991; Hahn et al. 1992). Of particular relevance to SV grafts is the study by Masood and colleagues (1997) who showed that ET-1 stimulates DNA synthesis in smooth muscle cells in a dose-dependent manner in organ culture of the human SV. Here, addition of ET-1 to denuded SV segments caused a significant increase in neointimal thickness and neointimal proliferation index when compared to controls. On the basis of their results the authors concluded that receptor antagonists may be of therapeutic value in the modulation of vein graft intimal hyperplasia. The following year this group demonstrated that the nonselective receptor antagonist, bosentan, and the ETB selective antagonist, BQ 788, significantly reduced neointima formation in organ culture of human SV, an effect that was not observed in the presence of the selective ETA antagonist, BQ 123 (Porter et al. 1998) (Fig. 1). Further involvement of ET-1 in neointimal thickening was shown in human SV using a dual endothelin converting enzyme (ECE)/neutral endopeptidase (NEP) inhibitor, a selective NEP inhibitor or vehicle (control). Here, segments of human SV were maintained in organ culture for 14 days with a dual ECE/NEP inhibitor, a selective NEP inhibitor or a vehicle control. SVs were then processed for immunostaining and measurement of neointimal thickness with conditioned media also being collected for enzyme-linked immunosorbent assay analysis. Neointimal thickness in the ECE/NEP-inhibited veins did not differ significantly from control segments but there was a significant augmentation in the NEP-inhibited segments. This result is consistent with an inhibition of ET-1 degradation. ECE immunostaining in the ECE/NEP-inhibited veins was reduced, while ET-1 staining was also present. Intense ET-1 expression was evident in the thickened neointima of NEP-inhibited veins as was significant ECE staining. Big ET-1 levels were identified in the ECE/NEP-inhibited veins, consistent with reduced ECE activity, with mature ET-1 still detectable in these segments. From these results it appears that potent and selective inhibitors of ECE are needed to evaluate the potential therapeutic benefits of blocking ET-1 biosynthesis rather than the use of dual inhibitors.

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These *in vitro* organ culture studies provide evidence for a role of ET-1 in promoting neointimal thickening in segments of human SV as well as the ability of ET receptor antagonists to reduce this effect. Of relevance to these studies is the reported distribution of ET-1, ETA and ETB receptors that has been examined in sections of thrombotic SV grafts. Here, using immunohistochemistry, positive ET-1 staining was associated with the thickened media and with components of the thrombotic occlusion, including proliferating vascular smooth muscle cells, collagen and regions of recanalization. The distribution of ETA receptor immunostaining was very similar to that for ET-1 with ETB receptor staining barely detectable except for that observed that was associated with endothelial cells at regions of recanalisation (Dashwood 2009) (Fig. 2).

In vivo studies

Various animal models of bypass surgery have been employed to assess the effect of ET receptor antagonists *in vivo*. Using a rabbit autologous SV to femoral artery graft model, Eguchi et al. (1997) showed that ET-1 developed the same force in the SV, the vein graft and the femoral artery. However, whereas Sarafotoxin developed force in the SV to the same extent as ET-1, it did not develop force in the femoral artery and only developed a slight force in the vein graft. Furthermore, using RT-PCR, the femoral artery expressed ETA receptor mRNA predominantly, the SV expressed both ETA and ETB receptor mRNA and, in vein grafts, the expression of ETB receptor mRNA was markedly reduced, but expression of ETA receptor mRNA remained unchanged. From these results the authors concluded that functioning ETB receptors and their mRNA are down-regulated when SVs are grafted into the arterial circulation and that these changes are part of adaptive responses associated with graft 'arterialization'.

A potential role for ET-1 in the development of intimal hyperplasia and atherosclerosis was suggested, based on the altered distribution and density of ET-1 and ETA receptor binding sites in both human and porcine blood vessels used as bypass grafts (Dashwood et al. 1993; Dashwood et al. 2004). Here, using *in vitro* receptor autoradiography, dense binding of $[^{125}I]$-ET-1 was observed to smooth muscle of all vessels examined, as well as to the vasa vasorum and regions of neovascularization of diseased vessels. This binding to the microvasculature may be of particular relevance since occlusion of the vasa vasorum by a close fitting collar in the rabbit carotid artery causes neointimal formation due to reduced transmural flow (Booth et al. 1989; Martin et al. 1991; Barker et al. 1994, 1995). Based on these observations it was therefore suggested that, apart from ET-1 causing neointimal thickening via an action on vascular smooth muscle cells of the SV, ET-1 acts on receptors located on the vasa vasorum of normal blood vessels and to regions of neovascularization

of atheromatous vessels implicating this peptide in the pathophysiology of atherosclerosis (Dashwood et al. 1993).

Perhaps the most persuasive data regarding the therapeutic potential for using endothelin receptor antagonists to reduce neointimal hyperplasia and improving SV graft performance in CABG is that generated from a porcine SV-carotid artery interposition graft model. Earlier studies, using this model, compared the degree of neointimal hyperplasia in SV into carotid artery grafts with control, ungrafted, SV (Angelini et al. 1992). Here, the time-course of a number of changes in the graft, compared to controls, were performed including morphometric assessment of medial and intimal size, cell number and density, endothelial morphology and cholesterol concentration. In addition, the effect of high pressure intraluminal saline distension was studied. In the first week after grafting, medial and intimal thickening occurred that was associated with an increase in cell number. Between 1 and 4 weeks after grafting further rapid medial and intimal thickening occurred. Also, distention to 600 mm Hg during surgical preparation of the SV for grafting resulted in lower graft patency after either 1 or 4 weeks. A number of time-related cellular and morphological changes were described establishing this as a useful pig bypass model for studying various strategies to reduce graft occlusion and improve graft performance. One particular novel finding, using this model, was that placement of a loose-fitting external stent around the SV graft reduces medial enlargement and hyperplasia (Violaris et al. 1993).

Since ET-1 had been established to possess both potent vasoconstrictor and cell proliferation properties, a study was undertaken on the pig model to investigate the potential role of ET-1 and its receptors in vein graft performance. Here, a combination of *in vitro* receptor autoradiography and immunohistochemistry was used to study changes in the distribution and density of ET-1, ETA and ETB receptor binding in grafted versus ungrafted SVs after 1 month (Dashwood et al. 1998). A number of interesting observations came out of this study, in particular: 1) autoradiography showed that vein grafts had a greater density of ETA compared to ETB receptors in both the tunica media and neointima; 2) Immunoreactive ET-1 was located on endothelial cells and throughout the neointima of the vein graft: 3) Dense ET-1 binding (to both ETA and ETB receptors) was also associated with microvessels and nerves in the adventitia within the graft (Fig. 3, Dashwood et al. 1998). On the basis of these observations it was concluded that ETA receptors may play a role in vein graft thickening at the medial and neointimal vascular smooth muscle cell level, whereas ETB receptors may play a role in microangiogenesis. Clearly, these data indicated that studies on the effect of ET receptor antagonists on the pathobiology of vein graft disease were warranted. Consequently, since ETA receptors were implicated in neointimal hyperplasia of experimental vein grafts, a follow up study was performed to investigate the effect of the selective ETA receptor antagonist, BSF 302146, on porcine vein graft thickening (Wan et al. 2004). SV-carotid artery interposition grafting was

performed in 4 groups of pigs with BSF 302146 administered orally at 3, 10, and 30 mg/kg for 4 weeks to groups of pigs and placebo administered to the control animals. Pigs were then anesthetized, the grafts were removed and histology performed on sections with morphometry carried out using computer-aided planimetry. In vein grafts from animals treated with BSF 302146, compared with (untreated) grafts from control animals, there were significant, dose-dependent, reductions in medial and neointimal thickness, an increase in luminal area, and a decrease in proliferating cells in the medial-intimal area (Figs. 3 and 4). This study therefore shows that the administration of BSF 302146 reduces graft thickening and promotes positive remodelling via an ETA receptor-mediated effect on vascular smooth muscle cell replication. It was therefore suggested, based on these results, that the administration of this ETA receptor antagonist might be therapeutically effective in preventing late vein graft failure in patients undergoing CABG.

A similar situation, where vascular damage occurs, is when angioplasty is used to restore myocardial blood flow in patients with coronary artery disease (Taggart 2007; Crişan et al. 2019). The damage to the endothelium and intimal layer caused by balloon inflation in many cases leads to neointimal hyperplasia and eventual restenosis of diseased vessels. The mechanisms underlying this process include vascular smooth muscle proliferation, collagen deposition and inflammation, very similar to those involved in vein graft failure. In an experimental model, balloon angioplasty of pig coronary arteries was shown to produce pronounced neointimal hyperplasia associated with vascular damage, in particular rupture of the internal elastic lamina, and a marked occlusion of the vessel lumen. The region of neointimal thickening observed 1 month after angioplasty exhibited dense [^{125}I] ET-1 binding, as identified by *in vitro* receptor autoradiography, as well as a predominance of ETA over ETB receptors (Dashwood et al. 1999). In those pigs administered the selective ETA receptor antagonist, LU 135252, neointimal hyperplasia was significantly reduced. Based on these results it was concluded that vascular smooth muscle cell proliferation and subsequent neointima formation is mediated predominantly via ETA receptors, underscoring the therapeutic potential of ETA receptor antagonists in reducing the degree of restenosis following vascular injury.

The knockout mouse provides a useful experimental model for the study of ET-1, ETA and ETB receptors. Indeed, the early study by Kurihawa et al (1994) showed that ET-1 knockout mice die of respiratory failure at birth as well as morphological abnormalities of the pharyngeal-arch-derived craniofacial tissues and organs. Since this publication various ET receptor knockout mice models have been used to study many of the diseases and pathological conditions described in the aforementioned reviews (Lüscher and Barton, 2000; Davenport et al 2016; Barton and Yanagisawa 2019, Dhaun and Webb 2019). Of particular relevance to the ET axis and neointimal thickening, more recent studies into the role of ET-1/ET receptors in vascular remodelling have been performed in genetically-modified mice. For example, using ETB receptor knockout mice, Murakashi et al (2002)

showed that the ETB system plays an antiproliferative role following injury of the common carotid artery. Further evidence for an involvement of the ET system in following vascular damage has been demonstrated, also in the injured carotid artery (Anggrahini et al. 2009). Here, transgenic mice were used where ET-1 exon 2 was flanked by two loxP sites. In these mice it was shown that endothelial ET-1 mediates vascular inflammation and neointima formation following injury of the common carotid artery. While these studies show a role for the ET system in vascular remodelling after injury, the focus on the carotid artery may be more relevant to the inflammation and neointimal formation that occurs following balloon angioplasty than vein graft occlusion. Ideally, a mouse venous bypass model is more suitable, such as that described by Zou et al . (1998) and Hu et al. (1999). However, such models require refined surgical expertise and have not so far been used in mouse knockout models or to examine the effect of ET receptor antagonists in vein graft occlusion.

External graft delivery

The effect of ETA receptor antagonists and SV graft patency has not received much attention over the last 20 years until the publication of a recent study showing that a bosentan-delivering sheath reduces neointimal hyperplasia in a rabbit bypass model (Xie et al. 2015). Here, a biodegradable external sheath was used to slowly release the dual ET receptor antagonist, bosentan. The bosentan-delivering sheath was placed around jugular vein into carotid artery grafts in a rabbit bypass model and compared with the effect of non-bosentan-containing sheaths and control grafts. After 8 weeks ultrasonography was performed on all groups and indicated that the change rate of the diameter of control vein grafts was much higher than that of sheathed or bosentan-delivering sheathed grafts (P < 0.01, respectively). The sheaths degraded completely 9 weeks after graft insertion and histological examination showed that the ratio of the area of intima-media to the area of lumen and the ratio of thickness of intima to the thickness of media of the bosentan-delivering sheaths were significantly lower than the control, non-sheathed group (P < 0.01) or the group surrounded by the non bosentan-delivering sheath (P < 0.05) (Fig. 5). From these results it was concluded that the degradable vascular external sheath with slow-release bosentan inhibits intimal hyperplasia and improves the shape of experimental vein grafts. Interestingly, fitment of the sheath alone (ie not containing bosentan) showed a significant reduction in neointimal thickening, an effect that is similar to that described previously using the porcine 'extent' model (Violaris et al. 1993). A number of mechanisms have been suggested that contribute to the beneficial effects of the pig external stent model, including protection of the vein graft against the effect of arterial haemodynamics, increased turbulence and shear and the promotion of adventitial neovascularisation (Jeremy et al. 2007). It seems, therefore, that the effects described in the rabbit bypass model by Xie et al. (2015) is likely due to a combination

of both the 'mechanical' influence of the external stent and the effect of bosentan blocking ET-1-mediated cell proliferation.

Vascular nerves

A novel finding from the pig vein graft model was the appearance of a time-dependent, increased, 'neoinnervation' in the grafts (Dashwood et al. 2000) (Fig. 6). At the short-term (1 month), large paravascular nerve bundles appeared that diminished in number at 6 months after vein graft surgery. While there was a sparse distribution of small paravascular nerves in control, ungrafted SVs, the density increased dramatically from 10.5 ± 1.7 per section in controls to 113.2 ± 102 in 1 month and 392 ± 76 in 6 month grafts. Positive ET-1 immunostaining was associated with microvessels within the nerve bundles as well as those microvessels that were adjacent to small nerve fibres. An interesting, and unexpected, observation in this study was the dense ET-1 immunostaining of the perineurium (Fig. 6), a structure associated with nerve maintenance and growth. The significance of this is unclear. However, regarding the vascular damage caused at SV harvesting, it is noteworthy that the perineurium creates a protective barrier where it plays a role in the nerve repair process following trauma, a situation that inevitably occurs when harvesting the SV for CABG (Peltonon et al. 2013).

A neurotransmitter/neuromodulator action of ET-1 was recognised almost since this peptide was discovered (Wong-Dusting et al. 1989; Reid et al. 1989; Wiklund et al. 1991). Certainly cerebrovascular nerves express ET-1 that may be related to both sensory and sympathetic neural components, according to observations following denervation in an *in situ* hybridisation study published by Milner et al. (2000*a*). ET-1-immunoreactive perivascular nerves have previously been revealed in animal (rat, capybara) and human cerebral vasculature (Loesch et al. 1998, 2005; Milner 2000*a,b*; Loesch and Burnstock 2002; Mickey et al. 2002). A combined immunocytochemical and electron microscope study of capybara basilar artery also showed the presence of nerve-associated ETA and ETB receptors in addition to those present on the endothelium, smooth muscle and Schwann cells (Loesch et al. 2005, 2013; also see Loesch and Dashwood. 2009) (Fig. 7). Perivascular nerves of human SV express NO/NOS (Tsui et al. 2002), where there is a rich presence of tyrosine hydroxylase (TH)-positive sympathetic plexus, as observed both at the confocal and electron microscope levels (Loesch and Dashwood 2009). It may be speculated, therefore, that at least a proportion of the ET-1-positive nerves in human SV are indeed of sympathetic nature (Loesch and Dashwood 2009). Whether such a possibility exists ie the colocalisation of ET-1 with sympathetic/noradrenergic nerves in human SV, and whether this would influence nerve activity, seems an interesting hypothesis to be examined in future studies. Several neuropeptides, including ET-1, regulate a wide variety of biological effects, including noradrenergic transmission and in particular neuronal NE uptake. A

recent review discusses the role for the interaction between ET-1 and NE in maintaining neurotransmission homeostasis, further suggesting that this interaction may represent a potential therapeutic target for various diseases, particularly hypertension (Vatta et al. 2015). This possibility is supported by the fact that ETA and ETB receptors are implicated in the mediation of contractile responses evoked by NE (Enouri et al. 2013).

Summary

From the studies described above it is clear that ET-1 has the potential to induce neointimal thickening in both *in vitro* and *in vivo* experimental conditions. ET-1 promotes neointimal hyperplasia in human SVs in culture, an effect that is reduced by dual and ETB-selective antagonists. In animal models, where experimental bypass grafts are performed, animals treated with dual, ETA- and ETB- receptor selective compounds have all been shown to be effective at reducing neointimal thickening. Although the organ culture studies provide a useful indication for the potential of ET receptor antagonists to reduce neointimal thickening in human SVs the experimental conditions do not reflect those of grafts in patients undergoing CABG. Here, the SV is subjected to varying degrees of trauma at harvesting as well as the post-implantation conditions where the vein is subjected to arterial haemodynamics, altered shear and turbulence, particularly at regions of anastomosis. All these conditions possess the potential to stimulate ET-1 release. In addition, ET-1 may be released in response to various aspects of tissue/cell damage such as the wound healing process and inflammation that occurs following CABG. There may be many potential sources of ET-1 release, from the (damaged) endothelium, vascular smooth muscle cells, vascular nerves, collagen, macrophages and other aspects of thrombus formation in occluded grafts.

If ET receptor antagonists are to be used to improve vein graft performance what strategies should be employed?

1) The most obvious might be for CABG patients to take a short course of the relevant antagonist post operatively (many will be taking aspirin) over an appropriate period.

2) SV explants, at harvesting, might be placed in storage solution containing ET receptor antagonist. In the operating theatre, during CABG, SV explants are stored in a variety of solutions such as blood, heparinised saline, or 'NO-donating' solutions (eg DuraGraft ®).

3) Local ET antagonist could be administered by the appropriate delivery system, such as a biodegradable external sheath.

Clearly, caution is advised if ET receptor antagonists are to be used to improve SV graft performance following CABG given the reported adverse side effects of some of these compounds (Lüsher and Barton, 2000; Dashwood and Tsui 2002; Davenport et al 2016; Barton and Yanagisawa 2019). Although such effects have been reported when administered systemically they may not occur if locally applied as in points 2 and 3 above. Where side effects may be caused when ET antagonists are administered systemically the question arises - what is more important, improving graft performance and restoring myocardial blood flow or experiencing potential minor side effects such as facial flush, headache, nausea, vomiting or constipation?

Conclusions

Coronary artery disease is a major cause of death worldwide with over 1 million patients undergoing CABG a year. The most commonly used vessel for myocardial revascularisation is the SV but a high percentage of these grafts fail with patients requiring reoperation. Graft failure is due to thickening of the intima, occlusion of the lumen and reduced myocardial blood flow. ET-1 is a peptide with potent vasoconstrictor and cell proliferation properties that is implicated in vein graft failure where it is suggested to play a role in graft spasm and occlusion. Endothelin antagonists have been shown to reduce neointimal hyperplasia in a variety of *in vitro* experimental studies as well as in *in vivo* animal bypass models. The safety and tolerability of many antagonists are now established and, given the current trend for repurposing of endothelin receptor antagonists, perhaps now is the time to test their potential to offer an adjuvant therapeutic approach for reducing SV graft failure in patients undergoing CABG. Potential starting points might be to assess the effect of 'tried and tested' antagonists on SV graft patency in patients following either systemic administration or *ex vivo* immersion in ET antagonist-containing storage solutions at the time of harvesting.

Conflict of interest

None

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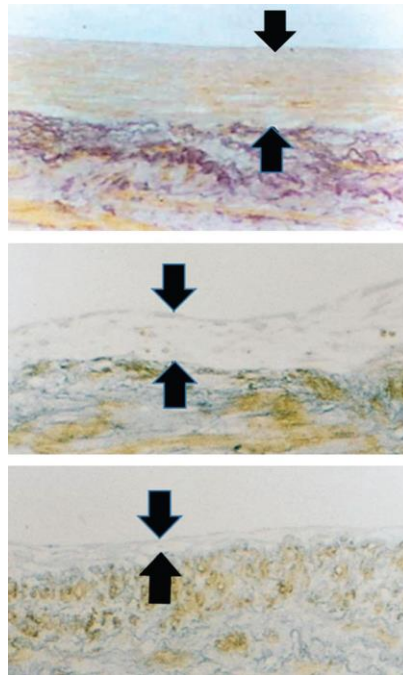
Figures and Legends

Figure 1. Effect of ET-1 and bosentan in organ culture of human saphenous vein. *Top panel*. Neointimal thickening produced by ET-1 in saphenous vein in culture for 7 days. *Middle panel*. Spontaneous neointimal thickening in human saphenous vein segment after 14 days in culture. *Lower panel*. Reduced neointimal thickening in human saphenous vein segment incubated in the presence of bosentan. The arrows indicate the region of neointimal thickening at the luminal surface. Modified from Porter et al J. Vasc. Surg. 28(4): 695-701 and Masood et al. Br J Surg. 997;84(4):499-503, with permission from John Wiley and Sons.
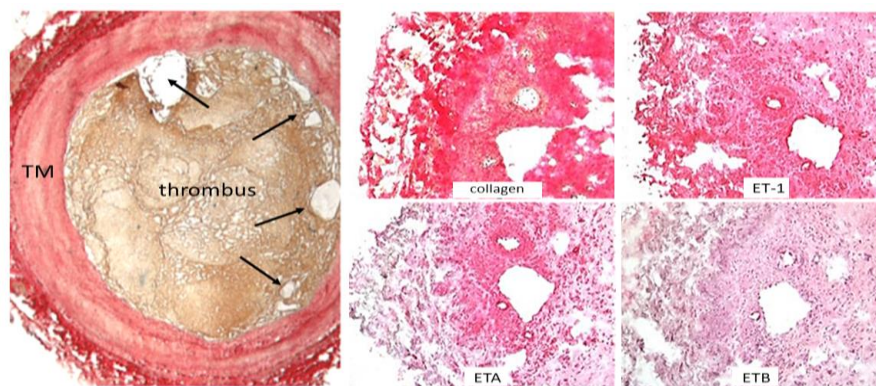
Figure 2. Thrombus formation in occluded human saphenous vein graft: ET-1 and ET receptor distribution. *Left panel*. Thrombus in an occluded saphenous vein graft showing regions of recanalization (arrows). *Right panel*. Immunohistochemical identification (originally red staining) of collagen, ET-1, ETA and ETB in a section of an occluded saphenous vein graft. The thrombus contains a high collagen and smooth muscle cell content displaying positive staining for ET-1 and ETA receptors and weak ETB staining. Modified from Dashwood 2009 Eur. J. Clin. Invest. 39 Suppl 2: 78-87, with permission from John Wiley and Sons.
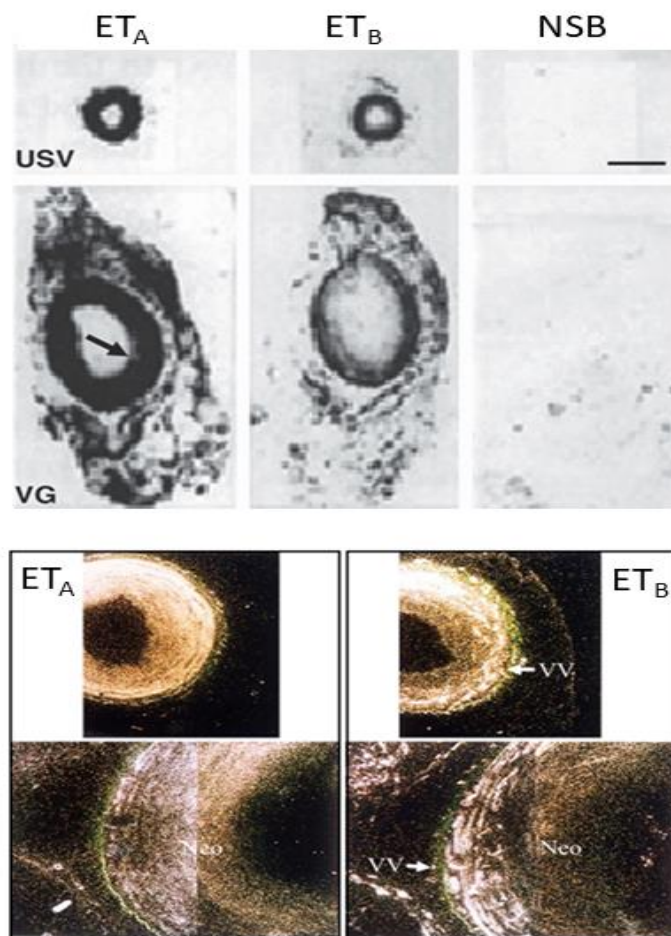
Figure 3. Distribution of ETA and ETB receptor binding sites in porcine saphenous vein graft. *Top panel.* Low resolution autoradiographs, generated on film, showing the distribution of ETA and ETB receptor binding to transverse sections of ungrafted (USV) and grafted (VG) porcine saphenous vein. In both grafted and ungrafted veins ETA>ETB binding. In the grafted vein there is increased binding to the tunica media and regions of intimal thickening (arrow) with ETA>ETB binding, particularly at regions of intimal thickening. *Lower panel.* High resolution autoradiographs generated using nuclear emulsion where binding is evident as white grains on a dark background. In the ungrafted veins ETA>ETB binding. At this resolution ETB can be observed within the media that is associated with the vasa vasorum (VV). In the graft ETA>ETB binding at the media and neointima (Neo). ETB<ETA binding at the media and neointima but there is an increased binding to the vasa vasorum as well as to perivascular regions that includes regions of 'neovascularisation' as well as perivascular nerves. Modified from Dashwood et al. 1998, Atherosclerosis. 137(2): 233-242 with permission from Elsevier.
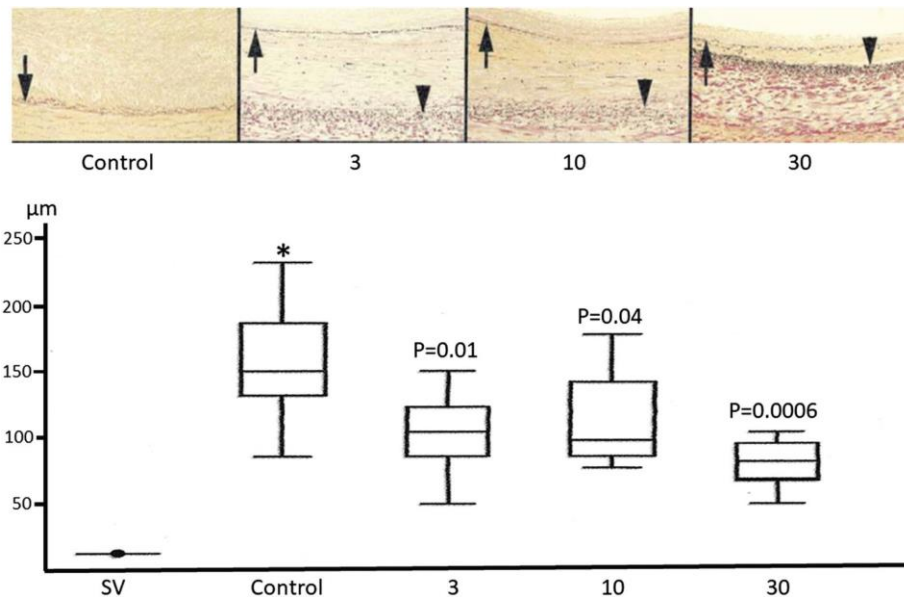
Figure 4. Effect of the ETA antagonist, BSF 302146, on neointimal formation in porcine saphenous vein grafts. *Top panels.* Effect of oral administration of the ETA antagonist, BSF 302146, on neointima formation in Elastin van Gieson–stained serial transverse sections of a representative control vein graft and grafts 4 weeks after administration of 3 mg, 10 mg and 30 mg/Kg of ETA antagonist. Arrows and arrowheads indicate the internal and external elastic lamina, respectively. *Lower panel.* Planimetric analysis of neointimal thickness in vein grafts from pigs to which BSF 302146 was administered orally once daily for 1 month before explantation. Data are expressed as medians and interquartile ranges with P values (n = 10 per group). Modified from Wan et al. 2004. J. Thorac. Cardiovasc. Surg. 127(5):1317-1322. Open Access article, Elsevier.
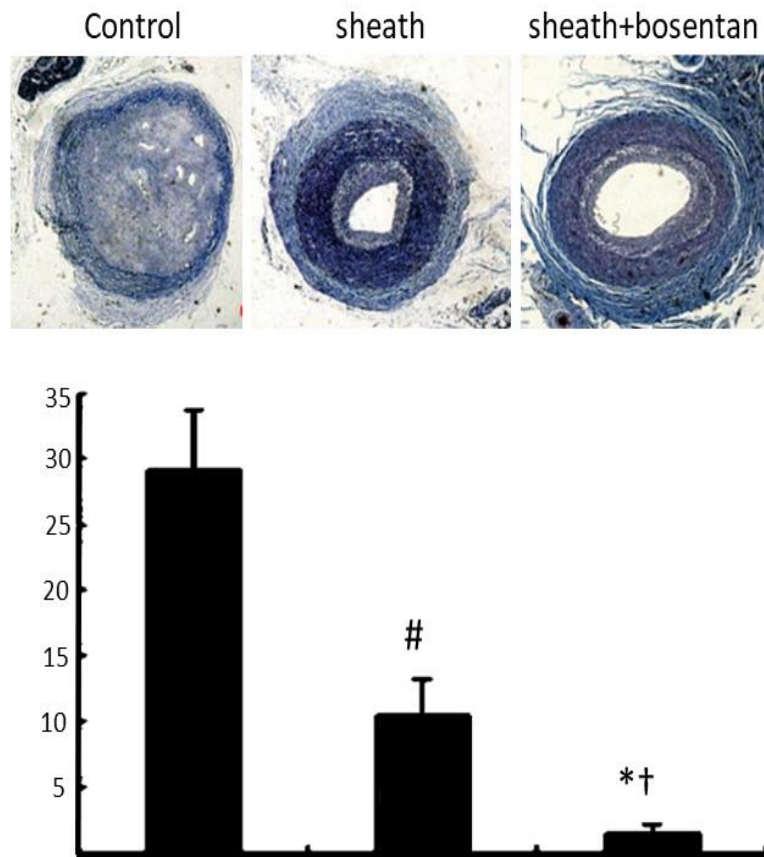
Figure 5. Effect of bosentan delivery on rabbit jugular vein graft structure. *Top panels.* Representative Alcian blue stained transverse sections of control (left), sheathed (middle) and sheath + bosentan (right). *Lower panel.* Histograms showing the results of histological examination (area intima-media/area lumen). * $P < 0.01$ vs Control; #$P < 0.05$ vs Control; †$P < 0.05$ vs Sheath + bosentan. Modified from Xie et al. 2015 Eur. J. Cardiothorac. Surg. 48(6):842-849. Open Access article, Oxford University Press.
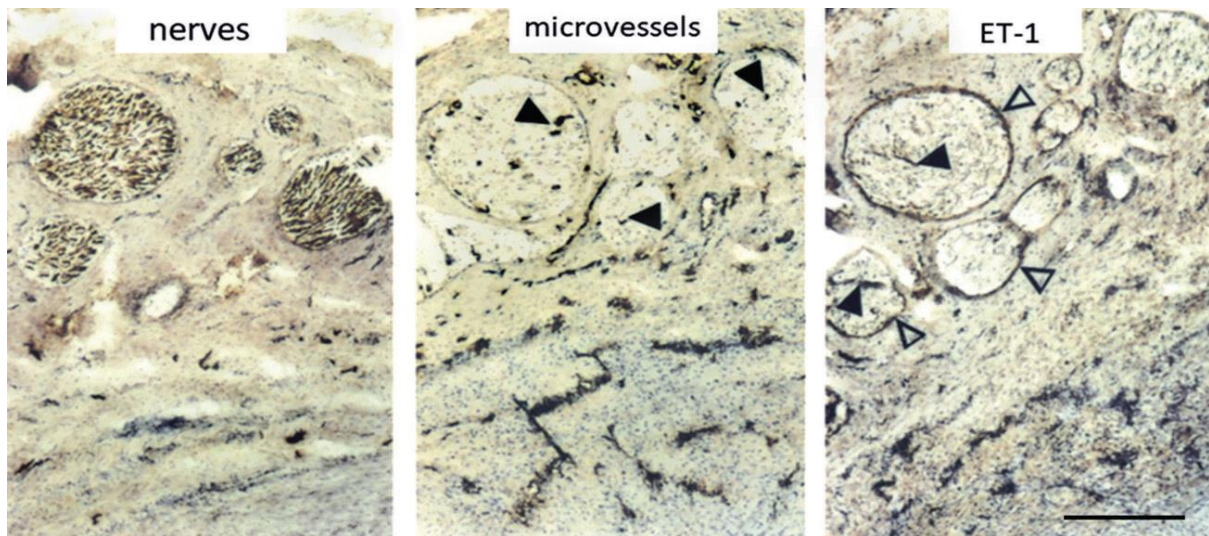
Figure 6. ET-1 immunostaining of nerves in porcine saphenous vein graft. *Left panel.* Paravascular nerve bundles and nerves identified in the adventitia of a 1-month vein graft. Brown immunostaining due to positive NF200 reaction. *Middle panel.* Adventitial and neural microvessels identified on an adjacent section using an antibody to von Willebrand's factor VIII indicated with closed arrowheads. *Right panel.* ET-1-like immunoreactivity on an adjacent section identified using an anti-ET-1 antibody. Positive immunostaining indicated with open arrows is associated with the perineurium and with neural microvessels (closed arrows). Scale bar = 20µm). Adapted from Dashwood et al. 2000 Atherosclerosis. 150(1): 43-53 with permission from Elsevier.
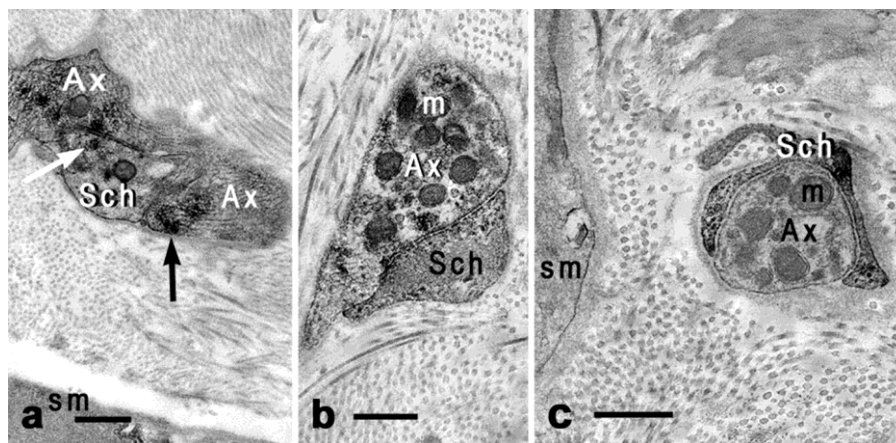

Figure 7. Capybara basilar artery perivascular nerves labelled (black precipitate) for ETA (a) and ET B (b, c) receptors (ExtrAvidin method). In (a) and (b) note axons (Ax) and Schwann cell profiles (Sch) displaying immunoreactivity (arrows) for ETA and ETB receptors, respectively. In (c) note ETB receptor-positive Schwann cell embracing an axon varicosity, which is immunonegative. Bars: 0.5 µm. Modified from Loesch et al. 2005 J. Mol. Histol. 36(1): 25-34 with permission from Springer Nature.