Somatostatin receptors and the potential use of Sandostatin[®] to interfere with vascular remodelling

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Abstract

Graft vessel disease (GVD) is a major cause of graft loss after the first year following transplantation. GVD is a complex, multifunctional process that involves immunological as well as non-immunological events such as ischaemia/reperfusion injury. An important target cell to interfere with the development of GVD is the smooth muscle cell (SMC). Somatostatin (SRIF) analogues have been shown previously to inhibit the proliferation of SMC *in vitro* and *in vivo*. We provide evidence that Sandostatin[®], an octapeptide SRIF analogue that is known to have anti-proliferative properties on SMC proliferation, inhibits vascular remodelling in a rat angioplasty model. Furthermore, in two allotransplantation models, Sandostatin[®] effectively interferes with the development of signs of chronic rejection/GVD. The role of the different SRIF receptor subtypes in chronic graft rejection is currently under investigation.

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Somatostatin (SRIF) receptor subtypes: pharmacology and receptor coupling

There are several endogenous ligands that bind to SRIF receptors, namely, SRIF-14, SRIF-28 and the cortistatins (1–4). Five distinct SRIF receptor (SRIF) genes have been described that encode receptor proteins of the seven transmembrane domain class within the superfamily of G protein-coupled receptors (5, 6). The five receptors show equally high affinity for SRIF and cortistatins (7, 8). All five receptors appear to couple to G proteins (6, 9, 10). Structural and functional information indicate that the SRIF receptors can be divided into two groups. The SRIF1 group (which comprises sst₂, sst₅ and sst₃ receptors) can be differentiated from the SRIF2 group (which comprises sst₁ and sst₄ receptors) by their selectivity of binding of short synthetic SRIF analogues, as well as on the basis of amino acid homologies (6, 11).

Many cellular effector proteins like adenylate cyclase, phospholipase C, calcium channels, potassium channels, Na⁺/H⁺ exchanger, protein tyrosine phosphatases, phospholipase A₂, mitogen-activated protein kinase or p53 were reported to be modulated by SRIF receptor subtypes (12). SRIF receptors are coupled to intracellular signal transduction cascades via various pertussis toxin-sensitive (G_i/G_o) and -insensitive G proteins (e.g. G_q, G₁₄, G₁₆). Depending on the cell type, the various SRIF receptor subtypes have been shown to couple to a diversity of transduction systems.

A number of short synthetic, metabolically stable peptide analogues have been studied for their relative affinities at the human recombinant receptors in radioligand binding studies (8, 13). The proclaimed selectivity of such ligands may, however, vary depending on the assay conditions and radioligands used (8, 14). In general, it is well accepted that sandostatin[®] and somatoline[®] show selectivity for two SRIF receptor subtypes, SRIF2 and SRIF5 (11).

SRIF analogues used to interfere with vascular remodelling

A remaining challenge to achieve long-term survival of transplanted organs is to prevent the development of chronic graft rejection (CR). The mechanisms involved are complex and multifactorial, hence multiple approaches have been explored to interfere with CR (15). A key event in the development of chronic graft failure is vascular changes in the graft, also described as graft vessel disease (GVD). These vascular changes include endothelial cell activation, media necrosis, neointima formation, and finally vessel occlusion resulting in late graft dysfunction. This process is very similar to the vascular remodelling observed during restenosis following angioplasty (16, 17). GVD appears to be triggered by a combination of alloantigen-dependent immune insult and alloantigen-independent factors such as donor age and ischaemia/reperfusion

Figure 1 Rats ($n = 12$ per group) were subjected to balloon injury of the left carotid artery and treated for 2 weeks with Sandostatin at an infusion rate of either 1 or 10 $\mu\text{g/kg}$ per h continuously delivered by a subcutaneously implanted minipump. On day 14 post-balloon injury the carotid arteries were subjected to *in vivo* MRI analysis (see text) prior to processing them for quantitative histology.

injury of graft endothelium. Both events lead to the activation/damage of graft endothelial cells. Subsequently, leukocytes invade the graft resulting in a local inflammatory response and growth factor-driven smooth muscle cell (SMC) migration and SMC proliferation into the intima, inducing graft vessel stenosis (16–19). Since migration and proliferation of SMC are key events in the development of vascular remodelling, pharmacological intervention with these processes appears to be a promising approach to prevent CR.

Based on their broad growth factor-inhibiting properties and their well-documented antiproliferative effects, SRIF analogues are promising candidates to reduce graft vascular remodelling (20). A number of preclinical rodent vessel as well as solid organ transplantation models have been described previously and these allow study of the mechanisms involved in the development of GVD (21). Short synthetic, metabolically stable SRIF analogues such as Sandostatin® (SMS 201–995, octreotide; Novartis Pharma AG, Basel, Switzerland) and Somatuline (BIM 23014, lanreotide; Henri Beaufour Institute, Washington, USA) have been shown to exert antiproliferative effects on SRIF receptor-expressing cells and to exhibit beneficial effects in some *in vivo* models of vascular remodelling/chronic rejection such as aorta allografting in the rat (21, 22).

Effect of SRIF analogues on SMC proliferation

In a previous paper by Grant *et al.* (23), it was shown that Sandostatin® as well as Somatuline® inhibit the insulin-like growth factor-I (IGF-I)- as well as the basic fibroblast growth factor (bFGF)-stimulated proliferation of human coronary artery SMC *in vitro*. In contrast, growth factor-stimulated SMC migration was not affected by either SRIF analogue (23). The authors concluded that by reducing SMC proliferation, SRIF analogues may exhibit beneficial effects in preventing restenosis after angioplasty. By using immunocytochemical techniques, growth factors such as IGF-I and bFGF have been localized in human coronary lesions. Studies by Lundergan *et al.* (24) indicated that the mechanism of action of angiopeptin to attenuate restenosis is not due to the effective inhibition of growth hormone (GH) and IGF-I but to autocrine/paracrine mechanisms that regulate cell replication. On the other hand, hypophysectomy was shown to inhibit arterial neointimal plaque formation in response to endothelial cell injury (25), indicating that endocrine factors such as GH release that are regulated by SRIF are involved in vascular remodelling.

To explore the potential use of Sandostatin® to interfere with vascular remodelling, we have tested its inhibitory effect in a rat carotid angioplasty model which involves the migration and proliferation of vascular SMCs. In this model, the left carotid artery of rats is ballooned, resulting in damage of the intimal layer and subsequent neointima formation. This process is similar to GVD. Sandostatin® was administered continuously to rats by minipumps at a rate of 1 and 10 $\mu\text{g/kg}$ per h for 16 days, resulting in plasma levels of approximately 1 and 10 ng/ml respectively. This regimen was chosen because it had been proven previously to exert potent antiproliferative effects in a number of rodent tumour models (26). As demonstrated in Fig. 1, histological examination revealed that Sandostatin® treatment resulted in a significant inhibition of neointima thickening following balloon injury. Importantly, parallel magnetic resonance imaging (MRI) measurements demonstrated that this inhibitory effect translates also into a gain in vessel lumen area (27). However, in line with observations made in a number of tumour models, the inhibitory effect of Sandostatin® on intimal thickening was incomplete in this model of restenosis. This would suggest the use of combined treatment regimes to further reduce vessel obliteration after angioplasty. RT-PCR experiments revealed the expression of low levels of *sst₂* mRNA in the rat carotid arteries, indicating the possibility of a direct, receptor-mediated antiproliferative effect of Sandostatin® in addition to the indirect effect via systemic or local growth factor inhibition. Expression of *sst₂* has previously been reported by Chen *et al.* (28), for the intimal surface of both normal and injured vessels using *sst₂*-specific antibodies.

Figure 2 Representative photomicrographs of Verhoeff elastin-stained sections which illustrate the degree of vascular remodelling in the mouse carotid artery loop model. (a) Placebo-treated (upper) and Sandostatin (SMS)-treated (lower) grafts. (b) Morphometric analysis of vascular remodelling using computer-assisted image analysis system in solvent-treated ($n=6$) and Sandostatin (SMS 201-995)-treated ($n=6$) groups ($P=0.005$) is shown. Values are means $\pm$ S.E.M.

In a clinical trial angiopeptin (Somatuline[®]) has been shown to significantly reduce restenosis, as well as clinical events after angioplasty (29).

Effect of Sandostatin[®] in a mouse carotid artery transplantation model

Transplant-associated arteriosclerosis is the leading cause of allograft failure after the first year following solid organ transplantation (30, 31). Multiple factors drive the process of vascular remodelling in allotransplantation. Thus, in addition to optimal immunosuppression, the development of novel therapeutic strategies to interfere with CR/GVD could be based on broadly acting growth factor inhibitors such as SRIF analogues. In fact, SRIF analogues have been demonstrated to be candidate compounds for treating GVD (32, 33).

We have investigated the potential protective effect of Sandostatin[®] in the mouse carotid artery loop transplantation model using B10.A(2R) (H-2^{h2}) mice as vessel donors and B10.BR (H-2^k) mice as recipients as

described previously (34). In this model, the donor carotid artery is transplanted paratopically as a loop onto the recipient carotid artery by end-to-side anastomosis. Sandostatin[®] or solvent control was delivered by Alzet minipump at a rate of $50 \mu\text{g/kg}$ per h. Carotid artery grafts were harvested at 30 days post-transplantation and subjected to quantitative histology to analyse the degree of vascular remodelling. In control animals, a massive neointima was formed in transplanted carotid arteries (Fig. 2). The mean neointimal area in control animals was measured to be $60.2 \pm 8.8 \times 10^3 \mu\text{m}^2$ ($n=6$). In contrast, in the group treated with Sandostatin[®] at $50 \mu\text{g/kg}$ per h from day 1 to day 28, neointimal formation was significantly reduced to a mean neointimal area of $1.34 \pm 4.3 \times 10^3 \mu\text{m}^2$ ($n=6$, $P=0.005$) (Fig. 2b). Histologically, the medial layer appeared to be well preserved and inflammatory cell infiltration was largely inhibited in the adventitia (Fig. 2a). These findings in the mouse carotid loop model confirm previous reports on the beneficial effects of SRIF analogues in rodent vessel transplantation models (35).

Effect of Sandostatin[®] in a rat kidney CR model

To further explore the potential use of Sandostatin[®] in preventing CR/GVD, the peptide was tested in a stringent rat kidney allograft chronic rejection model (36). Male Lewis (RT1^L) rats were grafted with a kidney from male Dark Agouti (DA) (RT1^a) rats. In this rodent solid organ transplantation model, acute rejection is prevented by 14 days of treatment with cyclosporine microemulsion Neoral (7.5 mg/kg per day; p.o. Novartis Pharma AG). Under these conditions, chronic rejection develops as early as 4–6 weeks post-transplantation. Continuous treatment with the long-acting release formulation of Sandostatin[®], Sandostatin[®] LAR (Novartis

Table 1 Effect of Sandostatin LAR in a rat kidney transplantation model (DA (RT1^a) $\rightarrow$ Lewis (RT1^L)). Data presented are number of animals in distinct groups of histology scores as described in the text.

Graft histology (score)	Placebo ($n=6$)	Sandostatin LAR (mg/kg)		
		3 ($n=6$)	10 ($n=5$)	30 ($n=6$)
Cellular rejection				
No	—	—	1	2
Slight–moderate	5	2	4	4
Moderate–end stage	1	4	—	—
Vessel histology				
Infiltration	1	1	2	4
Thickening	3	2	3	2
Narrowing	2	3	—	—

Pharma AG) at 3, 10 or 30 mg/kg injected on days – 2 and +40 after rat kidney transplantation improved signs of chronic rejection (cellular and vessel changes) dose-dependently as documented by histology (Table 1). Furthermore, a dose-dependent increase in organ perfusion was measured by MRI whereas in the placebo-treated controls the perfusion of the graft was reduced (36).

Conclusion

In conclusion, antiproliferative agents such as the SRIF analogue Sandostatin® (octreotide, SMS 201–995) are potential drug candidates for preventing/reducing restenosis and GVD. It remains to be investigated whether the clinically used SRIF analogues Sandostatin® or Somatuline® with their preferential affinity for sst₂ and sst₅ receptors or new analogues with a different binding profile for sst_{1–5} receptors are desired to interfere optimally with graft vessel remodelling. The role of the different SRIF receptor subtypes in chronic graft rejection is currently under investigation.

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