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In vitro investigation of integrin-receptor antagonist-induced vascular toxicity in the mouse

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ABSTRACT

An αVβ3 receptor antagonist (SB-273005) induced unique vascular lesions in the aorta of mice, but not other pharmacologically responsive species. Vascular smooth muscle cell (VSMC) necrosis was observed ~6 h postdose followed by VSMC loss with no evidence of hemorrhage/thrombosis, inflammation or damage to endothelium. Since direct drug-induced vascular toxicity is uncommon, involvement of VSMC–endothelial cell (EC) interactions was hypothesized. *In vitro* model systems of murine aortic VSMC and EC monocultures and cocultures were established and used to investigate the mechanism of toxicity. Incubation of cultures with SB-273005 within a dose range and timeframe comparable to *in vivo* studies, showed a concentration-dependent decrease in viability with increases in cytotoxicity for monocultures and VSMC/EC cocultures; however, VSMC monocultures responded at lower doses (were most sensitive) suggesting a direct effect on VSMC which is not mediated or enhanced through EC/VSMC interactions. Further studies revealed increased caspase-9 and caspase-3/7 activation in VSMC beginning as early as 0.5 and 1 h following treatment, respectively. These findings suggest SB-273005 causes direct chemical vascular toxicity in murine VSMC which involves apoptosis mediated through the intrinsic (mitochondrial) apoptotic pathway. To our knowledge, this is the first report to provide a link between VSMC apoptosis and treatment with an αVβ3 receptor antagonist.

1. Introduction

Integrins alphaV beta3 (αVβ3) and alphaV beta5 (αVβ5) are cell surface transmembrane receptors for extracellular-matrix (ECM) and proteins (Maubant et al., 2006) that are expressed on numerous cell types, including osteoclasts, vascular smooth muscle cells (VSMC) and endothelial cells (EC) (Kumar, 1998; Martin et al., 2002; Schwartz and Ginsberg, 2002). Besides mediating stable adhesion, integrins transmit signals to regulate cell survival, growth, motility, and remodeling of their extracellular environment (Coppolino and Dedhar, 2000; Hynes, 2002; Giancotti, 2000). They mediate a diverse array of biological events, including EC and VSMC cell-matrix adhesion, VSMC migration, angiogenesis, anoikis, apoptosis, and mechanotransduction of hemodynamic forces (Martin et al., 2002; Maubant et al., 2006; Schwartz and Ginsberg, 2002). As potential treatment agents, integrin antagonists are designed to disrupt the ECM involved in the pathogenesis of various diseases, including restenosis, diseases involving neovascularization, such as rheumatoid arthritis, tumor induced angiogenesis, metastasis and osteoporosis (Cacciari and Spalluto, 2005).

SB-273005 (Fig. 1), a non-peptide antagonist of the vitronectin integrin receptor αVβ3 (Lark et al., 2001; Miller et al., 2000) was previously in development for treatment of osteoporosis (Badger et al., 2001; Lark et al., 2001; Miller et al., 2000; Rehm et al., 2007). *In vitro*, SB-273005 was shown to inhibit bone resorption in cultures of human osteoclasts with an IC50 of 11 nM (Lark et al., 2001). *In vivo*, SB-273005 was efficacious in prevention and inhibition of bone loss in thyroparathyroidectomized and ovariectomized rats, respectively (Hoffman et al., 2002; Lark et al., 2001), but it was terminated from further development because of a unique vascular toxicity observed in the aorta of mice (Rehm et al., 2007). No such lesion was seen in rats, dogs or monkeys at similar or greater exposures suggesting possible species specificity.

Abbreviations: αVβ3, alphaV beta3; αVβ5, alphaV beta5; AA, allylamine; β-APN, β-aminoproprionitrile; DAPI, diamidino-2-phenylindole; DMEM, Dulbecco's Modified Eagle's Medium; DPBS, Dulbecco's phosphate buffered saline; EC, endothelial cells; ECGS, endothelial cell growth supplement; ECM, extracellular matrix; FBS, fetal bovine serum; FCS, fetal calf serum; HBSS, Hank's Balanced Salt Solution; RFU, Relative Fluorescence Units; RLU, Relative Light Units; VSMC, vascular smooth muscle cells.

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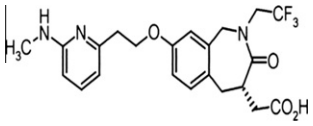

Fig. 1. Chemical structure of SB-273005 [(S)-3-Oxo-8-[2-[6-(methylamino)pyridin-2-yl]-1-ethoxy]-2-(2,2,2-trifluoroethyl)-2,3,4,5-tetrahydro-1H-2-benzazepine-4-acetic acid)], an αVβ3 vitronectin antagonist.

The lesion produced by SB-273005 in mice was dose-dependent (Table 1) and occurred rapidly (within 6 h) after oral administration and exhibited extensive degeneration (necrosis, hypertrophy, and collagen deposition) of VSMC in various segments of aorta (Rehm et al., 2007) with no evidence of vascular hemorrhage, thrombosis, or inflammation. No apparent damage to the endothelium was seen upon histological examination, although ultrastructural examination indicated changes in EC morphology as compared to controls. Upon drug withdrawal, no vascular regeneration or repair was observed. Vascular lesions were not observed with structurally-related antagonists, nor were such lesions observed in rats or monkeys at systemic exposures up to 100 times that achieved in mice (Table 2). Because the onset of the lesion occurred rapidly following a single oral dose of SB-273005 and because there was no histologic evidence of damage to the endothelium, the rapidity and extent of VSMC lesion development suggests a possible direct toxic effect on VSMC. An initial experiment examining the direct impact of SB-273005 *in vitro* indicated no toxic effect on murine VSMC but a dose-dependent toxicity to monkey VSMC (Rehm et al., 2007).

Direct chemical vascular injury has rarely been described, especially in mice. To our knowledge, the sweet pea toxin, β-aminopropionitrile (β-APN), or primary amine compounds, such as allylamine (AA), are the only two chemical substances that have been shown to induce direct chemical vascular injury in mice and rats (Boor et al., 1995). Acute administration of β-APN leads to inhibition of lysyl oxidase leading to interference with elastin and collagen crosslinking in aorta and connective tissue. Upon chronic administration, β-APN aortic aneurysms in aorta, and coronary and mesenteric arteries are observed. AA administration leads to subintimal proliferation and VSMC hypertrophy. Co-administration of both compounds for 10 consecutive days led to acute VSMC necrosis, disorganization of elastic fibers in large elastic arteries, and degeneration of mid-sized muscular arteries with no damage to endothelial cells, medial hemorrhage or inflammation. Acute effects following co-administration of β-APN and AA show striking similarities to SB-273005-induced aortic lesions; however, it took months following an off-treatment period for β-APN/AA-induced aortic lesions to show thinning of the media with loss of VSMC and disorganized elastic fibers rather than hours, as observed following SB-273005 treatment. Chronic administration of both compounds also led to aortic aneurysms that were not observed following either continued SB-273005 treatment for 3 months or upon drug withdrawal. Comparison of the morphological features of toxicity following coadministration of β-APN/AA to those of SB-273005 suggests the rapid SB-273005-induced VSMC toxicity may require VSMC communication and/or interaction with another essential component of the vasculature, namely EC.

Table 1
Systemic exposure following oral administration of various doses of SB-273005 to multiple species (GlaxoSmithKline unpublished data).

Species	Dose (mg/kg/day[a])	Ave AUC_{0-24} ($\mu g \cdot hr/mL$)
		Males and females
Mouse	30[b]	0.86
	100[b]	11.4
	300[b]	50.2
Rat	100[c]	111.0
Monkey	100[c]	172.2
Human	5 mg	0.03[d]
	20 mg	0.177[d]
	50 mg	0.64[d]
	100 mg	1.6[d]
	250 mg	4.5[d]
	500 mg	9.5[d]
	1000 mg	27.5[d]
	2000 mg	70.0[d]

[a] Dose is expressed as mg/kg/day unless otherwise noted.
[b] Aortic lesions observed.
[c] No effect level.
[d] Males only.

EC and VSMC are the major components of the vessel wall, and interactions between these cell types are known to play significant roles in maintaining the homeostasis of the structure and function of blood vessels (Chiu et al., 2003; Lavender et al., 2005). Cell–cell communication between these cell types also plays a fundamental role in vascular remodeling after injury (Chiu et al., 2003). EC, inflammatory cells and VSMC are involved in the pathogenesis of vascular pathologies (Milliat et al., 2006). VSMC migration, proliferation, and differentiation, which involve cell–cell communication between EC and VSMC, are critical processes involved in vascular pathologies, such as atherosclerosis and intimal hyperplasia (Milliat et al., 2006). EC–VSMC interactions involved in many of these vascular pathologies include signaling through adhesion receptors, which include αVβ3 and αVβ5 (Shattil and Ginsberg, 1997), the cellular targets of SB-273005. Due to the close contact and communication between EC and VSMC, vascular toxicants may exert effects on a specific cell type indirectly, through a disturbance of the normal cell–cell interactions in a given tissue.

The current study was undertaken to further explore the mechanism underlying the SB-273005-induced aortic toxicity, especially because the drug-induced direct chemical vascular toxicity is not common and rarely described in mice (Elwell and Mahler, 1999; Moyer et al., 2002; Rehm et al., 2007). To explore the potential dependence of EC on the mediation of SB-273005 induced aortic lesions, we sought to determine the sensitivity of EC and VSMC monocultures and cocultures to SB-273005 treatment, by investigating the effect of treatment on fundamental cellular processes, particularly viability and cytotoxicity. In addition, we have also attempted to dissect the temporal sequence and underlying mechanism of cell death noted, particularly in VSMC, following SB-273005 treatment.

We demonstrate that SB-273005 treatment results in a concentration-dependent decrease in viability with subsequent increases in cytotoxicity for both monocultures and VSMC/EC cocultures, with VSMC monocultures responding at lower doses suggesting a direct effect on VSMC that is not mediated or enhanced through EC/VSMC interactions. In addition, we demonstrate the involvement of apoptosis in SB-273005-induced VSMC toxicity. Furthermore, we also provide evidence suggesting the involvement of the intrinsic (mitochondrial) death pathway in the SB-273005-induced VSMC toxicity. To our knowledge, we are the first to report a link between apoptosis of VSMC and treatment with a non-peptide αVβ3 receptor antagonist.

2. Materials and methods

2.1. Test substance

SB-273005 (Fig. 1), a potent antagonist of the closely related integrins, human αVβ3 (Ki = 1.2 nM) and αVβ5 (Ki = 0.3 nM) and mouse αVβ3 (Ki = 0.19 nM), was obtained from GlaxoSmithKline Pharmaceuticals (King of Prussia, PA, USA).