The novel antiplatelet agent Revacept mitigates injury-induced vascular neointima hyperplasia

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Neointima hyperplasia is a critical component of restenosis, a major complication of angioplasty and related therapeutic procedures.Platelets may participate to vascular remodeling after vascular injury(Willerson et al.,1991) through the adhesion to vascular smooth muscle cells(VSMC) and the release of soluble factors[i.e. thromboxane(TX)A_2 and prostaglandin(PG)E_2, by the activity of cyclooxygenase(COX)-1, and growth factors, i.e. PDGF and EGF, stored in a-granules].Human platelet collagen receptor glycoprotein VI(GPVI) represents the major signaling receptor for collagen on platelets(Kehrel et al.,1998).Recently, it has been developed the novel antiplatelet agent Revacept, a fusion protein containing the extracellular domain of GPVI and the human immunoglobulin Fc domain.It binds to collagen and fibronectin in injured vascular tissue thus preventing platelet adhesion mediated by GPVI and possibly by other platelet receptors for collagen.Compared with antiplatelet agents currently in use, Revacept has the potential to reduce platelet activation without increasing bleeding(Ungerer et al.,2011). In a murine model of transluminal wire injury of the femoral artery (Roque et al.,2000) we studied the effect of Revacept administration on neointima formation by assessing intima-to-media(I/M) ratio.Moreover, we assessed the expression levels of protein markers of macrophage infiltration and vascular proliferation state by immunohistochemistry,i.e. CD68 and Ki67. Finally, we evaluated the occurrence of platelet activation in vivo by assessing the urinary levels of a major enzymatic metabolite of TXA_2, i.e. 11-dehydro-TXB_2(TXM), using immunoassay (Ciabattoni et al.,1987), before and after Revacept administration.C57BL/6 male mice received Revacept(at doses of 0.5,2,4mg/kg/day) or Fc (as control) via tail-vein injection, for 3days, before undergoing bilateral femoral artery denudation, and for other 7days after injury.Urine collections were performed at day 0(baseline), at day 3(before injury), at day 7 and day 32(i.e.,4 and 28 days after injury) for the assessment of TXM levels.At day 32, mice were sacrificed and femoral arteries were harvested.In paraffin-embedded femoral artery sections(7um), the I/M ratio was assessed as previously described (Zhang et al.,2013) and macrophage infiltration and vascular proliferation state were evaluated by immunohistochemistry using primary antibodies against CD68 and Ki67.After 4weeks from injury, I/M ratio of Fc-control groups averaged 1.28±0.13 while Revacept treatment caused a significant reduction of I/M ratio.At Revacept 0.5mg/kg, I/M ratio averaged 0.80±0.13 and it was significantly ($P<0.05$) lower than the value found in Fc-control mice.Higher doses of Revacept caused a comparable reduction of I/M ratio to the lower dose.Revacept (at each dose) reduced Ki67 expression and macrophage infiltration in the vascular tissue.Urinary TXM values significantly increased 3days after injury (212% of baseline values,pre-injury) and then returned to baseline values 28days after vascular injury. In Revacept-treatedmice, TXM values, assessed 3 and 28days after injury, were not significantly different from baseline values before vascular injury. In conclusion, the novel antiplatelet agent Revacept mitigated vascular neointima hyperplasia at 4weeks from injury. This effect was associated with the prevention of vascular injury-dependent increase of TXA_2 generation in vivo which suggests the involvement of the antiplatelet effect of Revacept in its inhibitory action on neointima formation. Revacept may represent a promising therapeutic strategy to prevent restenosis in patients with coronary artery disease treated with percutaneous transluminal coronary angioplasty and stent implantation.

Ciabattoni et al.(1987).*Biochim Biophys Acta* 918:293-297.
Kehrel et al.(1998).*Blood* 91:491-499.
Roque et al.(2000).*ATVB* 20:335-342.
Ungerer et al.(2011).*Circulation* 123:1891–1899.
Willerson et al.(1991).*Proc Natl Acad Sci USA* 88:10624-1068.