On the Ability of Imatinib Mesylate to Inhibit Smooth Muscle Cell Proliferation Without Delaying Endothelialization

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Statement of Purpose: Restenosis, the re-occlusion of a diseased vessel following a surgical intervention, is a major cause of failure of angioplasty, stenting, and bypass grafting with natural and synthetic vessels[1,2]. In healthy vessels, the endothelium exerts a control over smooth muscle cell (SMC) proliferation and migration. Unfortunately, revascularization procedures damage the endothelium of natural vessels and bypass vessels are completely devoid of endothelial cells. Many strategies have been developed to inhibit SMC proliferation and reduce intimal hyperplasia, yet most of the drugs tested thus far simultaneously inhibit endothelialization and do not selectively target SMCs. The ideal biological agent should have anti-proliferative effects on SMCs while preserving vascular healing and endothelialization so as to prevent late thrombosis. Imatinib mesylate is a specific inhibitor of three tyrosine kinase receptors, two of which, PDGF-R and c-Kit, are implicated in the pathogenesis of intimal hyperplasia[3,4]. In this study, we investigated *in vitro* the potential of imatinib mesylate to inhibit SMCs and its effect on ECs.

Methods: 72 h-proliferation assays were performed on human vascular smooth muscle cells (HVSMCs), human umbilical vein endothelial cells (HUVECs), bovine aortic smooth muscle cells (BAOSMCs) and bovine aortic endothelial cells (BAECs), with different concentrations of imatinib mesylate. The relative percentage of living cells was calculated with respect to a control without imatinib using the resazurin substrate. Western blot analyses of cleaved caspase 3 and PARP expression were performed on BAECs and BAOSMCs extracts to find out if imatinib induces apoptosis. The percentage of proliferating cells after 24h of incubation with or without imatinib was also determined by immunofluorescence. Cells were fixed and permeabilized prior to be labeled with an anti-PCNA.

Results and Discussion:

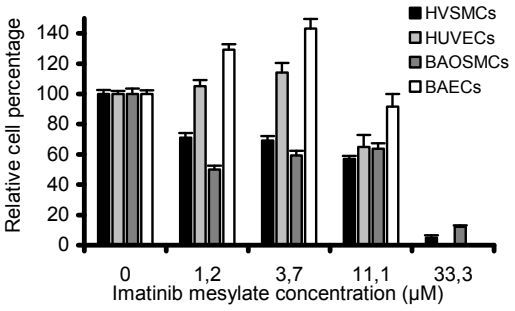

Figure 1. Relative cell percentage after 72 h of proliferation with imatinib mesylate compared to a control without drug.

The effect of imatinib mesylate on the proliferation rate of HVSMCs, HUVECs, BAOSMCs and BAECs was studied (Fig.1).For low concentrations (1.2 to 3.7µM), there was at least 30% less muscle cells when they were cultured with imatinib compared to a control without the drug. Surprisingly, there was 20 to 50% more ECs when cultured with imatinib. This effect was more prominent with the BAECs, although it did remain significant for the HUVECs at a concentration of 3.7 µM of imatinib. At higher concentrations, the drug lost its specificity and became toxic to all cells. These results were confirmed by immunofluorescence on BAECs and BAOSMCs co-culture (data not shown). In order to determine if the SMC's decrease at low doses of imatinib was due to apoptosis, western blot were performed to detect two late markers of apoptosis, cleaved caspase 3 and PARP (Fig. 2). No apoptosis was detected for BAOSMCs and BAECs cultured with 3.7 µM of imatinib.

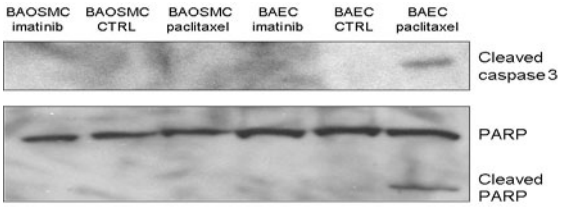

Figure 2. Western Blot analyses of cleaved caspase 3 and PARP expression in BAECs and BAOSMCs extracts untreated, treated with 3.7 µM imatinib, or 200 nM paclitaxel.

Since the decrease in SMCs could still be due to an inhibition of proliferation or cell death (necrosis), the percentage of proliferating cells was studied after 24 h of incubation with 0, 1.2 or 3.7µM of imatinib (Fig.3). As expected, the imatinib decreased the percentage of proliferating BAOSMCs from 93 to 63% for 3.7µM while BAECs displayed an increase in proliferating cells from 73 to 87%.

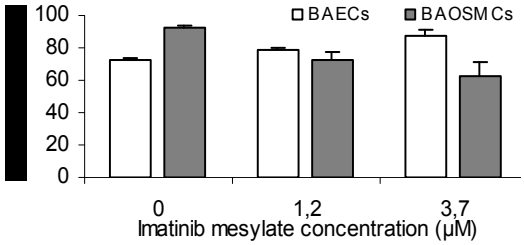

Figure 3. Percentage of proliferating cells after 24 h of incubation with or without imatinib mesylate

Conclusion: In light of these results, imatinib mesylate showed potential as a good candidate to inhibit intimal hyperplasia without delaying neo-endothelialization. This drug is particularly promising because it not only inhibits smooth muscle cells growth specifically but it also stimulates re-endothelialization.

References: 1. Bhoday, J. et al. *Current Vascular Pharmacology*, *4*, 269, 2006. **2.** Vamvakopoulos, J. E. et al. *J Vasc Res*, *43*, 184, 2006. **3.** Banai, S. et al. *Biomaterials*, *26*, 451, 2005. **4.** Sata, M. et al. *Nat Med*, *8*, 403, 2002.