Differential response to beta blockers of human endothelial cells from different sources

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Beta-blockers (β-B) play a major role in the treatment of several cardiovascular and systemic diseases. Intriguingly, a number of prospective studies have demonstrated that β-B possess anticancer effect and improve survival of cancer patients opening a new front of investigations on the role of these drugs in neoplastic diseases. The observation that propranolol exerts antiproliferative effect on infantile hemangioma (IH), a benign vascular tumor, also favors the hypothesis that β-B possess unexpected therapeutic properties. Although the treatment of IH with propranolol has shown spectacular results, the mechanisms are largely unknown and potential explanations include vasoconstriction, down-regulation of angiogenic growth factors and Matrix Metalloproteinases (MMPs) excretion, decrease in proliferation rates, and induction of apoptosis in endothelial cells (EC). Due to the wide range of pharmacologic activity of these compounds and their therapeutic implications in several diseases of different etiology, it is relevant to assess whether tissue specific ECs display different response to β-B.

The aim of the present study was to determine the differential effects of four β-B on hemangioma-derived (HemECs) and on human arterial-vein matched umbilical cord (HUAECs and HUVECs, respectively) EC *in vitro*.

Seven human umbilical cords from pregnancies to term were obtained. Samples of IH were from 9 children subjected to surgery. Following enzymatic digestion, HUVECs, HUAECs, and HemECs were cultured. The latter required an immune selection using CD31-microbeads. All cell lines were grown in EGM-MV plus 5% FBS. Passages 2 to 6 were used for the experiments.

ECs were treated with Propranolol hydrochloride (Prop), Atenolol (Ate), Metoprolol-tartrate (Meto) (25, 50, 75, 100, and 150μM), and Carvedilol (Carv) (1, 5, 25, and 50μM). After 48 hours exposure, proliferation was measured by Ki67 staining and apoptosis by TUNEL assay. The capacity to organize into tubule-like networks on Matrigel and migration by wound healing were evaluated after 24 hours. Oil Red O staining for lipid drops and TRITC-conjugated phalloidin for cytoskeletal organization were also detected by immunofluorescence. Western blot analysis was employed to identify potential molecular targets of β-B activity.

Prop, Ate and Meto at 150 μM concentrations as 50 μM Carv were cytotoxic and were not further investigated. All four β-B were able to inhibit EC proliferation in a dose-dependent manner. Prop and Carv induced apoptosis in ECs. No statistical differences were detected after lower doses of Prop and Carv nor for high doses of Ate and Meto. In contrast to several reports, ECs treated with high concentrations of all tested β-B retained the ability to organize into tubule-like networks on Matrigel.

Although a dose-dependent impairment in wound healing was observed with b-B, after exposure to Prop and Carv, wound repair was reduced in HemEC compared to HUVEC while corresponding HUAEC resulted more sensitive to the negative impact of β-B. Interestingly, high doses Meto did not exert significant impairment in healing process by all three EC lines.

Western blot analysis of CD146 and vascular endothelial growth factor receptor (VEGFR)-2 proteins was performed because recent studies shed light on the interplay between these two molecules and the cytoskeletal integrity of EC. No downregulation of CD146 and VEGFR-2 expression was detected in HemEC, HUVEC and HUAEC exposed to β-B. However, disarrayed cytoskeletal filaments were observed in all EC lines exposed to high dose β-B. Finally, Prop, Carv, Meto, and Ate induced in all EC lines dose-dependent morphologic changes consistent with autophagy.

Proliferation, migration and cytoskeletal organization of tissue specific endothelial cells are differentially modulated by β-B. These observations suggest that optimization of the wide clinical application of β-B agents requires patient and disease oriented approaches.