Differential effects of Raf inhibitors on upregulation of contractile endothelin B receptors in rat cerebral arteries after organ culture

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Cerebral ischemia results in upregulation of contractile endothelin B (ET_B) receptors in the smooth muscle wall of cerebral arteries contributing to further reduction in cerebral blood flow and tissue damage. Organ culture of isolated arteries has been shown to induce similar receptor changes as observed after cerebral ischemia and is therefore utilized as an *in vitro* method to study receptor changes in detail. The objective of the present study was to investigate and compare inhibitors of Raf, first signaling molecule of the MEK/ERK pathway, on ET_B receptor upregulation after organ culture. Rat middle cerebral arteries were incubated in serum-free medium for 24 h in absence or presence of Raf inhibitors ZM336372, GW5074, SB386023, SB590885 or LBT613. Contractile properties were evaluated in a wire myograph by cumulative addition of sarafotoxin 6c (S6c, ET_B receptor agonist) and endtothelin-1 (ET-1, here ET_A receptor agonist due to desensitization of ET_B receptors with S6c). Application of SB386032 significantly reduced ET_B receptor-mediated contraction in rat cerebral arteries after organ culture. LBT613 was also shown to have an effect, although a smaller reduction in the maximum contraction to S6c was observed. The other Raf inhibitors ZM336372, GW5074 and SB590885 did not affect ET_B receptor-mediated contraction significantly. The present study shows that application of Raf inhibitors could be used to attenuate upregulation of contractile ET_B receptors after organ culture. However, the nature of the inhibitor i.e. specificity and affinity for Raf and its subtypes (A-, B- and C-Raf) may explain the differential results on ET_B receptor-mediated contraction that was observed.