Clopidogrel Is an Inducer and a Potent Reversible Inhibitor of Cytochrome P450 3A4 In Vitro

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Background. Inter-individual variability in the response to clopidogrel has recently been described in patients undergoing percutaneous coronary intervention. A recent ex vivo study has suggested that: 1) clopidogrel is metabolically activated by cytochrome P450 (CYP)4A4, and 2) clopidogrel may be both an inducer and an inhibitor of CYP3A4. We hypothesized that the observed variability in the antiplatelet response to clopidogrel may be in part due to CYP4A4 induction via the nuclear pregnane X receptor (PXR) response element, and/or competitive inhibition of CYP3A4.

Methods. To test whether clopidogrel is capable of CYP4A4 induction in vitro, we used L5178Y tumor cell that express CYP3A4, and is inducible by classical PXR ligands. The cells were purchased from the American Type Culture Collection (Manassas, VA), plated, and cultured. Then total RNA was prepared and assayed for CYP3A4 mRNA expression using real-time polymerase chain reaction analysis in the presence of clopidogrel as compared to control. To test for CYP3A4 inhibition by clopidogrel, bacterially expressed human liver CYP3A4 in a reconstituted system was used. Using purified recombinant CYP3A4 the clopidogrel-dependent inactivation of the testosterone 6 beta-hydroxylase activity of CYP3A4 was investigated. The activity of CYP3A4 was measured by high pressure liquid chromatography. Incremental doses of clopidogrel were added to primary reaction mixtures containing purified CYP3A4.

Results. 1 µM and 10 µM clopidogrel induced CYP3A4 mRNA by 3.9 ± 0.2 and 8.7 ± 0.6 fold as compared to control. No mechanism-based or suicide inhibition of CYP3A4 by clopidogrel was observed (there was no significant loss of CYP3A4 activity following pre-incubation with clopidogrel and NADPH for 0, 5, 10, and 20 minutes). However, competitive inhibition of CYP3A4 by clopidogrel was observed at concentrations of 2.5 µM and 10 µM of clopidogrel with 61% and 68% of the catalytic activity of CYP3A4 inhibited, respectively.

Conclusion. Variability in clopidogrel response may be in part explained by the in vitro findings that clopidogrel is a CYP3A4 inducer and an potent reversible competitive inhibitor of human CYP3A4.

Insulin Enhances Cell Adhesion Molecule -1 Expression in Human Cultured Endothelial Cells: A Link to the Pathogenesis of Accelerated Atherosclerosis in Diabetes

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Hyperinsulinemia is a risk factor for atherosclerosis by mechanisms poorly understood. We assessed if insulin (I) can increase monocytes - endothelial interactions implicated in atherosclerosis. Human umbilical vein endothelial cells were incubated with I for 0 - 24 hours a tumor necrosis factor (TNFα 0.1 ng/mL), lipopolysaccharide (LPS 0.1 ng/mL), the p38mitogen activated protein(MAP) kinase inhibitor SB203580 (SB 0.1 - 20 µg/mL) and the phosphatidylinositol (PI3) - kinase inhibitor wortmannin (WT 10^8 to 10^10 M). Expressions of vascular cell adhesion molecule-1 (VCAM -1) or intercellular (ICAM-1) adhesion molecules, and E -selectin were assessed by enzyme immunoassay (EIA), flow cytometry, immunochemistry and northern analysis. I373 cell adhesion to endothelial cells was determined by a rotational adhesion assay. At pathological concentrations I induced surface expression of VCAM -1 but not ICAM -1 or E-selectin and potentiated the effects of TNF and LPS. 10^10 M WT increased I373 cell adhesion by 9.2 and 2.7 fold respectively, and markedly induced expression of VCAM-1 mRNA. In the absence of any cytokinity WT 10^-10 M potentiated the effect of I alone, while SB 1 µg/mL abolished this effect. In the presence of unstimulated control + I, pretreated with TNF -α, data lacking because of cytotoxicity. In conclusion I promotes VCAM -1 expression by a p38MAPK kinase pathway amplified by the PI3 -kinase block. This effect may contribute to atherosclerosis in hyperinsulinemic subjects.