O1 THE EFFECTS OF 25-HYDROXYCHOLESTEROL ON CYCLOOXYGENASE EXPRESSION IN HUMAN UMBILICAL VEIN ENDOTHELIAL CELLS: A POSSIBLE ROLE OF CHOLESTEROL ON CYCLOOXYGENASE EXPRESSION IN VASCULAR ENDOTHELIAL CELLS AT THE EARLY EVENT OF ATHEROSCLEROSIS

Duangporn Plasen[1], Pravit Akarasereenont[1], Kitirat Techatrisak[2], Sirikul Chotewuttakorn[1], Athiwat Thaworn[1]

[1]*Department of Pharmacology and* [2]*Department of Obstetric and Gynecology, Faculty of Medicine Siriraj Hospital, Bangkok 10700*

ABSTACT

25-hydroxycholesterol is the most abundant of cholesterol oxidative products which has been shown to be injurious to many types of mammalian cells including vascular endothelial cells and smooth muscle cells both *in vitro* and *in vivo*. Prostacyclin (PGI_2) is the most potent inhibitor of monocyte activation, platelet activation, secretion and aggregation. Cyclooxygenase (COX) is the rate-limiting step of PGI_2 synthesis. Constitutive and upregulated constitutive COX (COX-1) expression and inducible COX (COX-2) expression are important in PGI_2 production required for physiological and pathological defense of blood vessels. It is not known whether cholesterol oxidative products, one of the causes of atherosclerosis, effects on cyclooxygenase expression in vascular endothelial cells. Here we have investigated the effects of 25-hydroxycholesterol on cyclooxygenase expression in human umbilical vein endothelial cells (HUVEC). HUVEC was obtained from babies born to normal pregnancy and grown as standard technique. The medium in the third passage was replaced with fresh medium (control) and fresh medium containing 25-hydroxycholesterol (0.1, 1 and 10 μg/ml). Cells were then incubated at 37°C under 5% CO_2 concentration for variable periods of times (6, 12 and 24 hours). After which time, the release of 6-keto-$PGF_{1\alpha}$ (a stable metabolite of PGI_2) in medium was measure by enzyme immunoassay (EIA) technique and the remained cells were extracted to detect COX protein expression by Western blot technique. The effects of 25-hydroxycholesterol on cell viability were also investigated using MTT assay. All concentrations of 25-hydroxycholesterol (0.1, 1 and 10 μg/ml) and incubation times (6, 12, 24) used did not effect either cell viability or COX-1 expression. 0.1 μg/ml of 25-hydroxycholesterol up to 24h-incubation period could not induce COX-2 expression. However, at the higher doses (1 μg/ml for 24h and 10 μg/ml for 12 to 24h) incubation could induce COX-2 expression. The cyclooxygenase activities, measured from 6-keto-$PGF_{1\alpha}$ level in medium supplemented with 10 μM arachidonic acid, were also increased in HUVEC treated with 25-hydroxycholesterol at the dose of 10μM for at least 12 h and at the dose of 1 μM for at least 24h. Because of no change in COX-1 expression, the increase in COX activity should be due to the induction of COX-2. This may be the physiological defense of endothelial cells against vascular insults.