P5. THE EFFECTS OF EXOGENOUS ARACHIDONIC ACID ON CYCLOOXYGENASE ACTIVITY AND ISOFORM EXPRESSED IN ENDOTHELIAL CELLS

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ABSTRACT

Prostaglandin (PGs) have numerous cardiovascular and inflammatory effects. Cyclooxygenase (COX) is the first enzyme in the pathway in which arachidonic acid is converted to PGs, prostacyclin (PGI_2) and thromboxane (TX) A_2. PGI_2 is the major COX metabolite released from endothelial cells which participating in inflammation, atherosclerosis, thrombosis etcs. Endothelial cells can be activated by arachidonic acid (AA) and its metabolites. Here, we have investigated the effects of AA on the PGI_2 released from human umbilical vein endothelial cell (HUVEC) by measuring the production of 6-keto-$PGF_{1\alpha}$ (a stable metabolite of PGI_2) in the supernatant at various arachidonic acid concentrations and variable times of AA incubation. We also studied the effects of AA on COX protein expressed in HUVEC. HUVEC was obtained from babies born to normal pregnancy and grown in T25 flasks with endothelial cell (EC) medium supplemented with 10% fetal calf serum until confluent. The cells were then subcultured into 96-well culture plates, allowed to grow to confluent and replaced with fresh medium containing AA(0.1, 1, 10 and 20 μM). Cell were incubated at 37° C under 5 % CO_2 concentration in the CO_2-incubator for variable periods of times (5, 10, 20 and 30 minutes). After which time, 6-keto-$PGF_{1\alpha}$ in the supernatant medium was measured by using enzyme immunoassay (EIA). The remained cells were extracted to detect COX protein expression using specific antibody to COX-1 and COX-2. Cell respiration, an indication of cell viability, was assessed by the mitochondrial-dependent reduction of 3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide (MTT) to formazan. Either various concentrations (0.1-20 μM) or variable periods of times (5-30 minutes) of AA had any effect on cell viability. Control HUVEC without AA released undetectable amount of 6-keto-$PGF_{1\alpha}$ (< 3 pg/ml). At various concentrations of AA, HUVEC activated with exogenous AA could release higher amount of 6-keto-$PGF_{1\alpha}$ in a dose dependent manner. Interestingly, time of AA incubation did not affect the 6-keto-$PGF_{1\alpha}$ production at lower doses of AA (0.1, 1 and 10 μM). However, the higher concentration of AA (20μM) was trend to produce the higher 6-keto-$PGF_{1\alpha}$. Moreover, AA did not effect on COX protein expressed in HUVEC which were expressed COX-1 protein but not COX-2 protein. Thus, exogenous AA can increase PGI_2 released from HUVEC in a dose dependent manner but not time dependent. The increased PGI_2 release from HUVEC was from COX-1 function.