P3 THE EFFECTS OF COX- METABOLITES ON COX-2 INDUCTION IN IL-1β ACTIVATED ENDOTHELIAL CELLS.

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ABSTRACT

Objectives

To investigated the effects of COX-metabolites (PGI_2, PGE_2, $PGF_{2\alpha}$ and TXA_2) on COX-2 expressed in human umbilical vein endothelial cells (HUVEC) treated with IL-1β.

Materials & methods

Human umbilical vein endothelial cells (HUVEC) were obtained from babies born to normal pregnant women (HUVEC) and cultured in 96-well/6-well plates as standard techniques. Cells were grown to confluent and replaced with fresh medium containing no addition, IL-1β alone, COX-metabolites alone and IL-1β plus COX-metabolites (0.001, 0.01, 0.1 or 1 μg/ml) for 24h. Then, the medium was removed and replaced with fresh medium containing arachidonic acid (10 μM for 10 min). After which time, the medium was collected to measured COX activity by the production of 6-keto-$PGF_{1\alpha}$ (stable metabolites of PGI_2) using enzyme immunoassay. The remained cells were extracted and detected COX isoform expression by using immunoblotting.

Results

PGI_2, PGE_2, $PGF_{2\alpha}$ or TXA_2, did not affect on basal COX activity in untreated HUVEC (24h incubation). Untreated HUVEC contained COX-1 protein but not COX-2 protein. When HUVEC were treated with IL-1β (1 ng/ml for 24h), COX activity and COX-2 protein was increased in a dose dependent manner. The increased COX activity in IL-1β (1 ng/ml) treated HUVEC was inhibited with PGE_2 (0.03, 0.3 or 3 μM), but not PGI_2, $PGF_{2\alpha}$ or TXA_2 in a dose dependent manner. Similary, COX-2 protein expression in IL-1β treated HUVEC was also inhibited with PGE_2, but not PGI_2, $PGF_{2\alpha}$ or TXA_2 in a dose dependent manner.

Conclusion

These results suggested that PGE_2, but not PGI_2, $PGF_{2\alpha}$ or TXA_2 is a key in feedback regulation of COX-metabolites produced in HUVEC.