PHARMACOLOGICAL ACTIVITY OF SELECTIVE COX-1 AND COX-2 INHIBITORS IN ENDOTHELIAL CELLS

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The housekeeping prostanoid produced by the endothelium is PGI_2, which notably contributes to the maintenance of the physiological, anti-adhesive and anti-thrombotic properties of this "organ". Although a large body of data suggests that COX-1 is the constitutive source of endothelial prostacyclin, there is now a general agreement that the majority of PGI_2 in vascular endothelium is generated by COX-2.
We evaluated how selective COX-1 and COX-2 inhibitors affect PGI_2 production in human endothelium i) under basal conditions, ii) in the presence of IL1βiii) in the presence of different concentration of substrate and iv) upon modulation of hydroperoxide tone. We firstly demonstrated that resting endothelial cells, grown in the presence of 5% FCS express both protein isoforms nearly to the same extent of about 2 ng per µg total cell protein. Selective inhibitors of either COX-1 (SC560, FR122047) or COX-2 (SC236) concentration-dependently (1-300 nM) reduced basal prostacyclin production. However, at concentrations where their selectivity is preserved, as confirmed using the whole blood assay, each inhibitor decreased PGI_2 synthesis by more than 70%. In addition, both COX-1 inhibitors SC560 (COX-1 IC_{50} 9 nM, COX-2 IC50 6.3 µM) and FR122047 (COX-1 IC_{50} 0.028 µM, COX-2 IC_{50} 65 µM) were effective in reducing prostacyclin production after stimulation of HUVEC with □□□□□ interleukin 1β̃ In order to explain this evidence, we evaluated the presence of COX-1/COX-2 heterodimers in HUVEC showing their presence by immunoprecipitation and Western blot analysis.
The ability of selective COX inhibitors to interfere with PGI_2 production was also evaluated in the presence of exogenous substrate or hydroperoxides, as may occur under pathological conditions. In the presence of 10 µM arachidonic acid (AA), both selective COX inhibitors concentration-dependently decreased prostacyclin production, but SC236 lost most of its inhibitory activity in the presence of higher concentrations of arachidonic acid. Furthermore the inhibition of PGI_2 production was completely lost when 12-HPETE was added to the exogenous AA to mimic the occurrence of significant oxidative stress.
Altogether these results imply that the mechanism by which PGI_2 production is carried out under basal conditions involves COX-1/COX-2 etherodimers and that under "crisis" conditions COX-1 significantly contributes to the final production of PGI_2.