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[PC3-7] [04/18/2003 (Fri) 09:30 - 12:30 / Hall P]

ROLES OF NADPH OXIDASE AND COX-2 IN UVB-INDUCED MMP EXPRESSION IN HaCaT HUMAN KERATINOCYTES.

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Ultraviolet (UV) irradiation is well known to cause human skin aging and skin cancer through activation of matrix metalloproteinases (MMPs) which are responsible for the degradation of collagen, an extracellular matrix component. However, the molecular mechanisms of UV-induced MMP expression are yet to be defined. In this study, we investigated signaling molecules involved in UV-induced MMP expression in HaCaT human keratinocytes. UVB irradiation increased the generation of superoxide radical and lipid peroxidation. In addition, UVB enhanced MMP-1, MMP-2, and MMP-9 expression in a time-dependent manner. Pretreatment with N-acetylcysteine, trolox, general antioxidants, or DPI, neopterine and apocynin, NADPH oxidase inhibitors reduced VUB-induced superoxide radical release and expression of MMP-1, MMP-2, MMP-9, whereas malonate, hydroxyurea, rotenone, NMMA and FCCP had no effect on either ROS release nor MMP expression. In addition, UVB increased COX-2 expression. Furthermore, indomethacin, a nonselective COX-2 inhibitor reduced UVB-induced MMP-1, MMP-2, and MMP-9 expression and ROS production. These data suggest that NADPH oxidase and COX-2 play important roles in VUB-induced MMP level though reactive oxygen species generation.

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The efficient Erythropoietin expression system in Chinese Hamster Ovary cells by introduction of urea cycle enzymes

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The efficient EPO (Erythropoietin) expression system in Chinese Hamster Ovary (CHO) cells was devised through the removal of ammonium ion accumulated in the media by introducing urea cycle enzymes. Previously, we developed CO5 cell by transfecting the carbamoly phosphate synthase (CPS) and ornithine transcarbamoylase (OTC) into the EPO expressing CHO cell, IBE. This study showed that CO5 had 17%, 18%, and 21% higher cell viability and 17%, 20% and 21% lower ammonia concentration per cell than IBE on condition that media were changed on every 24hr, 48hr and 72hr, respectively. Also, productivity of EPO in CO5 was 97%, 116% and 125% higher than that in IBE when media were changed on every 24hr, 48hr and 72hr, respectively. These results showed that CO5 expressing CPS and OTC was superior to IBE on cell viability, reduction of ammonia concentration and EPO production on condition that media were changed with 72hr interval than that with 24hr and 48hr interval. These results support that reduction of ammonium ion accumulated in the media is proportional to EPO production and cell viability. EPO were purified by DEAE and affinity column to examine carbohydrates moiety of glycoprotein quantitatively. Comparisons of glycosylation of EPO purified from IBE and CO5 is currently in progress by IEF(isoelectric focusing gel) and quantitative sialic acid analysis.