protection against oxidative stress

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A substantial body of evidence indicates that reactive oxygen intermediates (ROIs) are implicated in pathogenesis of diverse human diseases, including cancer, diabetes and neurodegenerative disorders. Oxidative stress induced by ROIs often causes cell death via apoptosis that is regulated by a plenty of functional genes and their protein products. In the present work, we have investigated the role of bcl-2 in protecting against oxidative death induced by hydrogen peroxide in cultured rat pheochromocytoma (PC12) cells. When PC12 cells were treated with hydrogen peroxide, they underwent apoptotic death as determined by characteristic morphological features and internucleosomal DNA fragmentation. Hydrogen peroxide treatment also led to the decreased mitochondrial membrane potential, increased Bax expression, activation of caspase-3, and cleavage of poly(ADP-ribose)polymerase. Transfection with the anti-apoptotic bcl-2 rescued PC12 cells from oxidative cell death caused by hydrogen peroxide. Addition of NF-κB inhibitors, pyrrolidine dithiocarbamate or L-tosylamido-2-penetyl chloromethylketone to the media aggravated hydrogen peroxide-induced PC12 cell death. PC12 cells overexpressing bcl-2 exhibited relatively high constitutive DNA binding and transcriptional activities of NF-κB, compared with the vector-transfected control cells. In addition, sustained NF-κB activation was observed in the bcl-2 overexpressing cells, which was accompanied by the constitutive activation of extracellular signal-regulated kinase 1/2. However, bcl-2 overexpression did not cause any significant alterations in the activity of either c-Jun N-terminal kinase and p38 mitogen-activated protein kinase. The ectopic expression of bcl-2 caused elevated level of cellular glutathione and expression of γ-glutamate-cysteine ligase and catalase, which were inhibited by NF-κB inhibitors. These results suggest that NF-κB plays a role in bcl-2-mediated protection against hydrogen peroxide-induced apoptosis in PC12 cells through augmentation of antioxidant capacity.

[OC1-2] [2003-10-11 10:30 - 10:45 / ASEM Hall Meeting Room 208]

Celecoxib inhibits phorbol ester-induced expression of cyclooxygenase-2 and skin-tumor promotion in mouse skin: p38 and AP-1 as possible molecular targets

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Celecoxib, the selective cyclooxygenase-2 (COX-2) inhibitor, has recently been reported to reduce the formation of polyps in patients with familial adenomatous polyposis. This specific COX-2 inhibitor also protects against experimentally induced carcinogenesis, but molecular mechanisms underlying its chemopreventive activities remain largely unresolved. In the present work, we found that celecoxib inhibited 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced expression of COX-2 in female ICR mouse skin when applied topically 30 min prior to TPA as determined by both immunoblot and immunohistochemical analyses. In another study, celecoxib attenuated the DNA binding activity of activator protein-1 (AP-1) through suppression of c-Jun and c-Fos expression in TPA-treated mouse skin. Under the same experimental conditions, celecoxib inhibited both the catalytic activity and phosphorylation of p38 mitogen-activated protein (MAP) kinase in mouse skin. While the p38 inhibitor SB203580 blocked TPA-mediated AP-1 activation and c-Jun expression, the MEK1/2 inhibitor U0126 was not inhibitory despite suppression of c-Fos expression in mouse skin. Furthermore, SB203580 markedly blunted COX-2 expression induced by TPA. An anti-tumor promoting activity of celecoxib was examined in a two-stage mouse skin carcinogenesis model with 7,12-dimethylbenz[a]antharcene as an initiator and TPA as a promoter. Topical application of celecoxib (10 mmol) prior to each TPA treatment reduced the multiplicity of papillomas by 32% at 18 week. All the tumors analyzed exhibited elevated COX-2 levels compared with surrounding normal tissues or vehicle-treated control skin. The COX-2 expression was found to be repressed in the papillomas from celecoxib-pretreated mice. Taken together, down-regulation of COX-2 by blocking activation of p38 MAP kinase and AP-1 may account for the anti-tumor promoting activity which celecoxib exerts in mouse skin tumorigenesis.

[OC2-1] [2003-10-11 10:45 - 11:00 / ASEM Hall Meeting Room 208]

Characterization of Muations in DNA Gyrase and Topoisomerase IV Involved in