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

Resistant Mutants to DW-286a, a Novel Quinolone Antibiotic, in Streptococcus pneumoniae

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Quinolone resistance in Streptococcus pneumoniae is related to mutations in the DNA gyrase and topoisomerase IV genes. DW-286a displayed potent activity against S. pneumoniae C9211 (MIC, 0.015 μg/ml) compared with gemifloxacin (MIC, 0.06 μg/ml). This study was performed to analyze the ability of DW-286a to cause resistance development in S. pneumoniae and to establish whether DNA gyrase or topoisomerase IV is primary target. DW-286a resistant mutants of S. pneumoniae C9211 were generated by stepwise selection at increasing drug concentration. Sequence analysis of PCR products from the mutant strains was used to examine the quinolone resistance-determining regions (QRDR) of GyrA and GyrB proteins of DNA gyrase and the analogous regions of ParC and ParE subunits of the DNA topoisomerase IV. First-step mutants exhibiting low-level resistance had an alteration in GyrA at Ser-83, with Ser-83 to Tyr or Phe being observed. Second-step mutants had mutations in GyrA at Ser-83 to Tyr and in ParC at Ser-79 to Tyr at the same time. Third-step mutants displaying more high-level resistance were found to have additional change in GyrA at Glu-87 to Lys. Moreover, fourth-step mutants had additional mutations in ParC at Asp-83 to Asn, together with other mutations. No changes in GyrB, and ParE were observed in these mutants. Complementary genetic and biochemical studies revealed that GyrA and ParC are dual targets for DW-286a in S. pneumoniae, and resistance to DW-286a in S. pneumoniae occurs in vitro at a low frequency. To determine the level of expression of PmrA, a putative efflux pump of S. pneumoniae, we performed the analysis of QC-RT PCR. There were distinguishable increases in the expression of efflux pump (PmrA), so this phenotype indicated the presence of efflux mechanism of resistance in these mutant strains.

[OC3-1] [2003-10-11 11:00 - 11:15 / ASEM Hall Meeting Room 208]

Caspase-3-mediated cleavage of Cdc6 induces nuclear localization of truncated Cdc6 and apoptosis

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We show that Cdc6, an essential initiation factor for DNA replication, undergoes caspase-3-mediated cleavage in the early stages of apoptosis in HeLa cells and SK-HEP-1 cells induced by etoposide, paclitaxel, ginsenoside Rh2, or TRAIL. The cleavage occurs at the $SEVD^{442}/G$ motif and generates an N-terminal truncated Cdc6 fragment (p49-tCdc6) that lacks the carboxy-terminal nuclear export sequence (NES). Cdc6 is known to be phosphorylated by cyclin A-Cyclin A-dependent kinase 2 (Cdk2), an event that promotes its exit from the nucleus and probably blocks it from initiating inappropriate DNA replication. In contrast, p49-tCdc6 translocation to the cytoplasm is markedly reduced under the up-regulated conditions of Cdk2 activity which is possibly due to the loss of NES. Thus, truncation of Cdc6 results in an increased nuclear retention of p49-tCdc6 that could act as a dominant negative inhibitor of DNA replication and its accumulation in the nucleus could promote apoptosis. Supporting this is that the ectopic expression of p49-tCdc6 not only promotes apoptosis of etoposide-induced HeLa cells but also induces apoptosis in untreated cells. Thus, the caspase-mediated cleavage of Cdc6 creates a truncated Cdc6 fragment that is retained in the nucleus and induces apoptosis.

[OG-1] [10/11/2003(Sat) 11:15-11:45/ Asem Hall 203]

Patient counseling of over-the-counter drugs to enhance the pharmacist's role

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This presentation is to enhance the pharmacist's role in Over-The-Counter(OTC) drug selection and patient counseling for diversification of pharmacy management after the separation of prescribing and dispensing practice in Korea. Self-medication by OTC drugs may be viewed as one element of the broader self-care treatment. The patient may use a OTC drug to manage a minor ailment, a process that may be supported by counseling from a pharmacist. Pharmacists involved in self-medication decisions have a greater involvement with patients and an