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