Schedule-dependent interactions between paclitaxel and etoposide in human carcinoma cell lines in vitro

Yasuhiko Kano · Miyuki Akutsu · Kenichi Suzuki
Kiyoshi Mori · Yasuo Yazawa · Saburo Tsunoda

Abstract Clinical studies of paclitaxel in combination with etoposide against solid tumors have been carried out. The combination schedules used in these studies are different. We studied the cytotoxic effects of paclitaxel with etoposide against four human cancer cell lines in vitro to determine the optimal schedule of this combination at the cellular level. Cells were exposed simultaneously to paclitaxel and etoposide for 24 h or sequentially to one drug for 24 h followed by the other for 24 h, after which they were incubated in drug-free medium for 4 and 3 days, respectively. Cell growth inhibition was determined by an MTT reduction assay. The effects of drug combinations at concentrations producing 80% inhibition (IC\textsubscript{80}) were analyzed by the isobologram method of Steel and Peckham. The cytotoxic effect of paclitaxel and etoposide was cell line- and schedule-dependent. Simultaneous exposure to paclitaxel and etoposide for 24 h produced additive effects in the lung cancer cell line A549 and ovarian cancer PA1 cells, and antagonistic effects in the breast cancer cell line MCF7 and colon cancer W1Dr cells. Sequential exposures to paclitaxel followed by etoposide and vice versa produced additive effects in all four cell lines. These results suggest that maximum cytotoxic effects can be obtained with sequential administration, but not simultaneous administration, of paclitaxel and etoposide. These findings may have important clinical implications for this combination.

Key words Paclitaxel · Etoposide · Drug combination · Isobologram

Introduction

Paclitaxel is a new antimicrotubular agent with significant activity against a variety of solid tumors, including lung cancer, breast cancer, and ovarian cancer [2, 10, 12, 24, 38]. Unlike vinca alkaloids, this drug promotes the polymerization and stabilization of tubulin to microtubules, thereby inhibiting the dynamic reorganization of the microtubule network required for mitosis and cell division [31, 46, 47]. Cells in the S phase are most sensitive to paclitaxel and accumulate in the G\textsubscript{2} /M phase [37]. The primary dose-limiting toxicity of paclitaxel is myelotoxicity, mainly neutropenia. Mild peripheral neuropathy has been reported, and hypersensitivity reactions and cardiac arrhythmia have been rarely observed [11, 19, 51].

Etoposide is a semisynthetic podophyllotoxin derivative with a broad spectrum of activity against a wide variety of malignancies, including leukemia, lymphoma, germinal tumor, and lung cancer [44]. Etoposide is thought to exert its main cytotoxic action by stabilizing cleavable complexes formed by DNA and the nuclear enzyme topoisomerase II [34]. Cells in the S phase are most sensitive to etoposide, and etoposide-treated cells accumulate in the late S/G\textsubscript{2} phase [35]. The dose-limiting toxicity of etoposide is myelotoxicity, mainly neutropenia. Nonhematological toxicities are mild to moderate. Despite its use as a single agent and in a number of combination chemotherapy regimens, the optimal use of etoposide remains unknown.

The combination of paclitaxel and etoposide is rational, based on the marked antitumor activity of both agents against a variety of solid tumors, their different...
cytotoxic mechanisms, and the different toxic profiles other than that of neutropenia. There has been interest in identifying the optimal schedules for this combination, but considerable controversy exists over whether these drugs produce synergistic, additive, or antagonistic effects when given together or sequentially [7, 9, 14, 20, 30, 40, 50]. The combination of paclitaxel and etoposide in various schedules has been used for the treatment of lung cancer, breast cancer, colon cancer and a variety of other cancers [5, 6, 18, 33, 41, 43, 49]. We therefore conducted an in vitro investigation of the effects of paclitaxel and etoposide in combination against human cancer cell lines in various schedules. The analysis of the effects of the drug-drug interactions was carried out by the isobologram method proposed by Steel and Peckham [48].

Materials and methods

Cell lines

The experiments were conducted with four human carcinoma cell lines: the non-small-cell lung cancer cell line A549, the breast cancer cell line MCF7, the ovarian cancer cell line PA1, and the colon cancer cell line WiDr. These cells were obtained from the American Type Culture Collection (Rockville, Md.) and maintained in 25-cm² plastic tissue culture flasks containing RPMI-1640 medium (Grand Island Biological Co., Grand Island, N.Y.) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Grand Island Biological Co.) and antibiotics. The cells used were free of mycoplasma infection. A549, MCF7, and PA1 cells were bcl-2-positive.

Drugs

Paclitaxel and etoposide were provided by Bristol-Myers Squibb Japan Co. (Tokyo) and Nihon Kayaku Co. (Tokyo), respectively. Paclitaxel was dissolved in dimethyl sulfoxide (Sigma Chemical Co., St Louis, Mo.), and etoposide was dissolved in RPMI-1640. The drugs were diluted with culture medium. The final concentrations of dimethyl sulfoxide in the medium was less than 0.1%, and it had no effect on cell growth inhibition in our study. Since the cytotoxic levels of paclitaxel and etoposide in clinical practice are generally maintained for more than 10 h, a 24-h exposure to paclitaxel and etoposide was used in the present experiments.

Inhibition of cell growth by combinations of anticancer agents

Exponentially growing cells were harvested with trypsin (0.05%/EDTA (0.02%) and resuspended to a final concentration of 5.0 × 10⁵ cells/ml in fresh medium containing 10% FBS and antibiotics. Aliquots of the cell suspensions (100 µl) were dispensed using a multichannel pipette into the individual wells of a 96-well tissue culture plate with a lid (Falcon, Oxnard, Calif.). Each plate had one eight-well control column containing medium alone and one eight-well control column containing cells but no drug. Four plates were prepared for each drug combination schedule in each cell line. The cells were reincubated overnight in a humidified atmosphere containing 5% CO₂ at 37 °C to allow attachment.

Sequential exposure to paclitaxel first followed by etoposide and vice versa

After cell attachment, aliquots of medium containing 10% FBS (50 µl) and solutions of paclitaxel (or etoposide) (50 µl) at different concentrations were added to individual wells. The plates were then incubated under the same conditions for 24 h. The cells were washed twice with culture medium containing 1% FBS and fresh medium (200 µl) was provided. The cells were then incubated again for 4 days.

MTT assay

Viable cell growth was determined by a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described previously [27]. For all cell lines examined, we were able to establish a linear relationship between the MTT assay and the cell number within the range used in these experiments.

Isobologram method

The dose-response interactions between paclitaxel and etoposide at the point of IC₅₀ were evaluated by the isobologram method of Steel and Peckham [48]. The theoretical basis of the isobologram method and the procedure for making isobolograms have been described in detail previously [26, 28, 48].

Based upon the dose-response curves of paclitaxel and etoposide, three isoeffect curves were constructed (Fig. 1). If the agents