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Cisplatin Down-regulates Topoisomerase I Activity in Lung Cancer Cell Lines

KEISUKE AOE[1,2], KATSUYUKI KIURA[1], HIROSHI UEOKA[1], MASAHIRO TABATA[1], MASAKAZU CHIKAMORI[1], HIROYUKI KOHARA[1,2], MINE HARADA[1] and MITSUNE TANIMOTO[1]

[1]*Department of Internal Medicine, Okayama University Medical School, Okayama;*
[2]*Department of Respiratory Medicine and Clinical Research, National Sanyo Hospital Respiratory Disease Center, Yamaguchi, Japan*

Abstract. *Many clinical studies have reported that irinotecan has reproducible antitumor activity against lung cancer. Both cisplatin and SN-38 are key drugs in the treatment of lung cancer, and their combination is one of the most promising regimens available. Using lung cancer cell lines, ABC-1 and SBC-3, we examined the cytotoxic effect of the schedule, as well as the effect of cisplatin on topoisomerase I activity. Cytotoxicity was determined by MTT assay. ABC-1 or SBC-3 cells were incubated with or without various concentrations of both drugs in 96-well microplates for 72 or 96 hours in a humidified 5% CO_2 atmosphere at 37˚C. Synergism was evaluated by median-effect plot analysis and a combination index isobologram method by Chou and Talalay. After ABC-1 or SBC-3 cells had been exposed to 10 μM cisplatin for one hour, topoisomerase I activities were determined by supercoiled-DNA relaxation assay. Synergism was observed in ABC-1 and SBC-3 cells when cisplatin was given first, followed by SN-38 (7-ethyl-10-hydroxycamptothecin) and cisplatin. Topoisomerase I activity decreased at 1-2 hours after exposure to cisplatin and recovered gradually after 4-5 hours of cisplatin exposure in both ABC-1 and SBC-3 cells. Accordingly, pretreatment with cisplatin will have an impact on the sensitivity to SN-38.*

Irinotecan (7-ethyl-10- [4-(1-piperidyl)-1-piperidino] carbonyloxy-camptothecin) is a water- soluble camptothecin analog, which reversibly inhibits DNA topoisomerase I (1). Topoisomerase I inhibitors as single agents show excellent activity to a wide variety of tumors, especially lung cancer and colon cancer (2). In addition, topoisomerase I inhibitors may also interfere with DNA repair and enhance cytotoxicity when combined with DNA-damaging agents (3). In preclinical studies, the combination of irinotecan and cisplatin showed synergistic effects in several tumor cell lines (4, 5).

Clinical studies on the combination of irinotecan and cisplatin have been undertaken in a variety of tumors (6-10). In the majority of clinical trials, irinotecan was administered first followed by cisplatin, although the schedule dependency of this combination is still controversial. For the development of combination chemotherapy, the schedules as well as the appropriate combinations should be preclinically investigated. Thus, we studied the schedule dependency of the combination with cisplatin and SN-38, which is an active metabolite of irinotecan, and the effect of cisplatin on topoisomerase I activity.

Correspondence to: Keisuke Aoe, MD, PhD, Department of Respiratory Medicine and Clinical Research, National Sanyo Hospital Respiratory Disease Center, Ube, Yamaguchi 755-0241, Japan. Tel: +81-836-58-2300, Fax: +81-836-58-5219, e-mail: keisukeaoe@mtf.biglobe.ne.jp

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Materials and Methods

Chemicals. Cisplatin and 7-ethyl-10-hydroxycamptothecin (SN-38) were provided by Bristol-Myers Squibb K.K., Tokyo and Yakult Honsha Co., Ltd., Tokyo, Japan, respectively. SN-38 was dissolved in dimethyl sulfoxide. 3-[4,5-dimethyl-thiazol-2-yl]-2, 5-diphenyl tetrazolium bromide (MTT) was purchased from Sigma Chemical Co., St. Louis, MO, USA.

Cell lines. SBC-3 (JCRB0818) and ABC-1 (JCRB0815) cells were established in our laboratory from patients with small cell lung cancer and adenocarcinoma of the lung, respectively (11-13). The cell lines were maintained in a humidified atmosphere with 5% CO_2 in air at 37˚C, in RPMI 1640 medium (GIBCO BRL, Grand Island, NY, USA) containing penicillin (100 U/mL) and streptomycin (100 μg/mL) and 10% fetal bovine serum (FBS, GIBCO BRL) (RPMI-FBS).

Cytotoxicity assay. The cytotoxic activities of each drug and combination effects of two drugs were determined by MTT assay (14) with a slight modification, as described previously (13, 15). Briefly, 100 μl aliquots of RPMI-FBS containing serial concentrations of chemotherapeutic agents and cells (ABC-1: 3000 cells/well, SBC-3; 2000 cells/well) were plated in 96-well

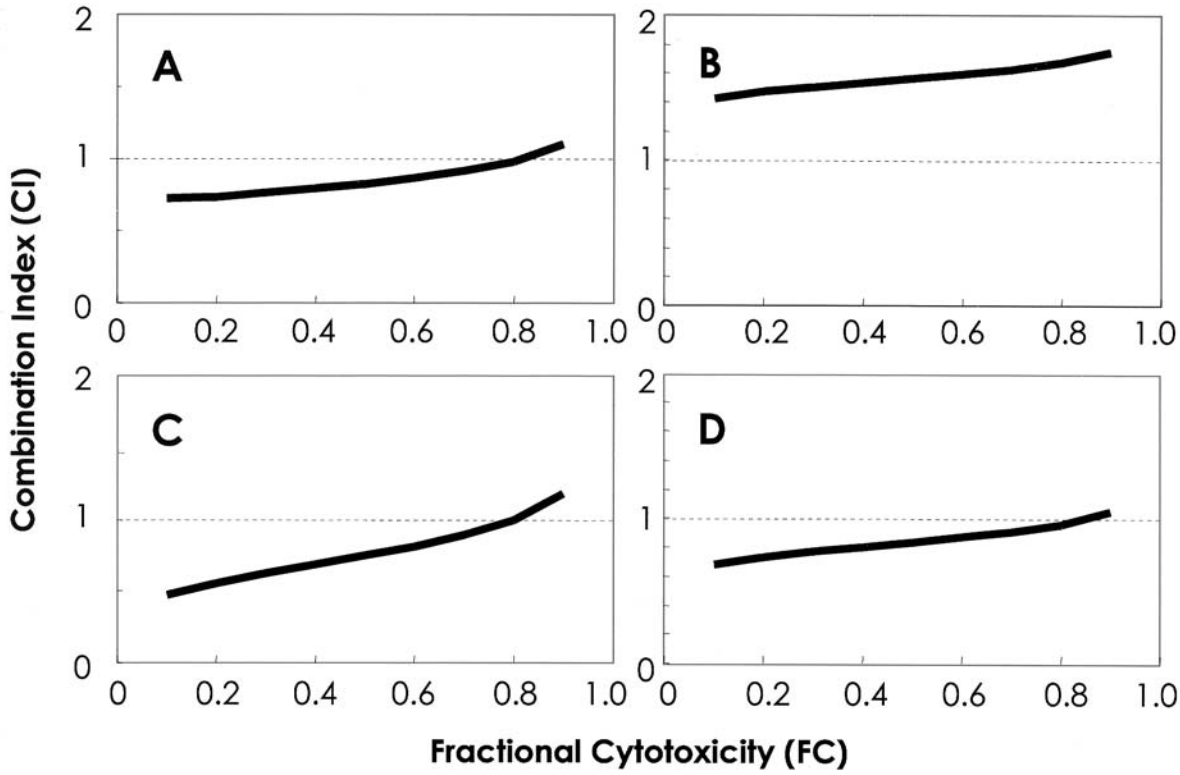

Figure 1. *A, B, C and D: Combination index isobolograms in combination with cisplatin and SN-38. Combination index (CI) < 1; synergy, CI=1; summation, CI>1; antagonism. A. ABC-1 cells: cisplatin -> SN38. B. ABC-1 cells, SN38 -> cisplatin. C. SBC-3 cells, cisplatin -> SN38. D. SBC-3 cells, SN38 -> cisplatin.*

flat-bottomed microplates and incubated at 37˚C for 72 or 96 hours in a humidified atmosphere with 5% CO_2 in air. Cisplatin or SN-38 was administered for 24 hours before concurrent exposure. Then, the cells were concurrently exposed to cisplatin and SN-38 for 72 hours. MTT formazan was dissolved in fresh isopropanol. The absorbance at 560 nm was measured using a microplate reader (Model 3550; Bio-Rad Laboratories, Richmond, CA, USA). Percent growth inhibition was defined as percent absorbance inhibition within appropriate absorbance in each cell line. All experiments were repeated at least twice. The combination effects were determined by median-effect plot analysis and combination index isobolograms (16-18). Combination index (CI) < 1 indicates synergy, CI=1, summation and CI>1, antagonism.

Preparation of nuclear extract. ABC-1 or SBC-3 cells were exposed to cisplatin at a final concentration of 10 μM for one hour. After washing, the cells were resuspeneded in fresh RPMI-FBS. After 1, 2, 3, 4, 5, 6 and 14(15)-hour incubations with cisplatin, the cells were collected and a nuclear extract from the ABC-1 and SBC-3 cells was prepared, as described previously (15). The total protein concentration was determined by Bio-Rad protein assay kit.

Topoisomerase I activity assay. Nuclear extract (0.4 μg) was added to the topoisomerase I reaction mixture containing 10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 150 mM NaCl, 0.1% BSA, 0.1 mM spermidine, 5% glycerol and 0.75 μg supercoiled DNA plasmid at a final volume of 20 μl. Following incubation at 37˚C for 15 minutes, the reaction was terminated by adding 5 μl of stopping buffer (final concentration; 1% Sarkosyl, 0.025% bromophenol blue and 5% glycerol). The reaction products were analyzed by electrophoresis on 0.8% agarose gel using a TBE buffer (89 mM Tris-HCl, 89 mM boric acid and 62 mM EDTA) at 1 V/cm, stained by ethidium bromide (0.5 μg/ml) and photographed using a short wavelength UV lamp.

Results

Cytotoxicity assay and combination effects. The combination index isobolograms of the schedule-dependent interaction between cisplatin and SN-38 in ABC-1 and SBC-3 cells are shown in Figures 1A, B, C and D. In ABC-1 cells, the combination index showed lower than 1.0 in a wide range of fractional cytotoxicity when cisplatin was given first followed by SN-38 and cisplatin (Figure 1A). On the other hand, the combination index was higher than 1.5 in a wide range of fractional cytotoxicity when SN-38 was given first followed by SN-38 and cisplatin in ABC-1 cells (Figure 1B). This combination showed synergism only when cisplatin was given first in ABC-1 cells.

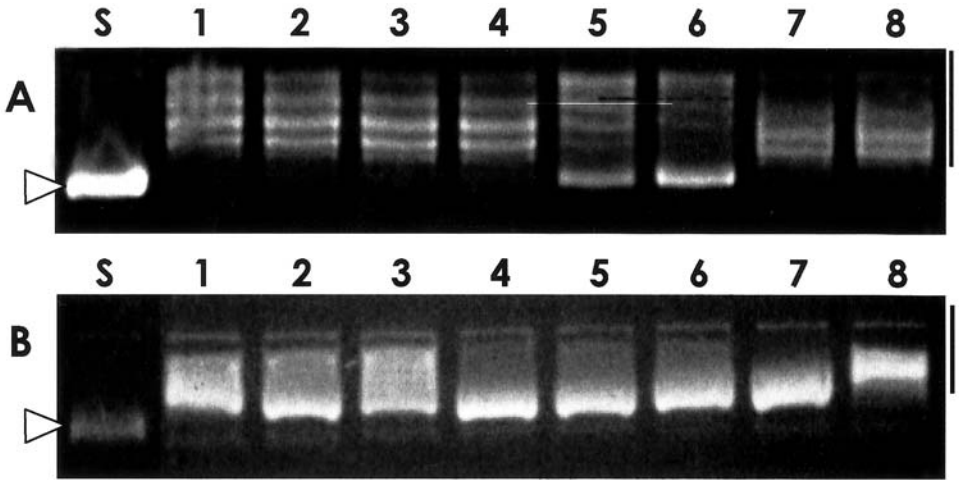

Figure 2. *A and B: Topoisomerase I activity of ABC-1 cells after cisplatin exposure. Supercoiled DNA (lane S), time after cisplatin exposure: 15h (lane 1), 6h (lane 2), 5h (lane 3), 4h (lane 4), 3h (lane 5), 2h (lane 6), and 1h (lane 7), and no exposure (lane 8). B. Topoisomerase I catalytic activity of SBC-3 cells after cisplatin exposure. Supercoiled DNA (lane S, white arrowhead), time after cisplatin exposure: 14h (lane 1), 6h (lane 2), 5h (lane 3), 4h (lane 4), 3h (lane 5), 2h (lane 6), and 1h (lane 7), and no exposure (lane 8). Supercoiled DNA and relaxed DNA are indicated with white arrowheads and bars, respectively.*

In SBC-3 cells, the combination index was lower than 1.0 in a wide range of fractional cytotoxicity without association of exposure sequence (Figures 1C and D). The combination index was lower when cisplatin was given first followed by SN-38 and cisplatin.

Topoisomerase I activity assay. Untreated ABC-1 and SBC-3 cells showed clear topoisomerase I activity, because relaxed DNA bands and no supercoiled DNA were observed (lane 8 in Figures 2A and B). As shown in Figure 2A, the topoisomerase I activity of ABC-1 cells did not change at one hour after cisplatin exposure (lane 7). Supercoiled DNA bands appeared at 2-3 hours after cisplatin exposure (lanes 5 and 6) and disappeared gradually after 4-5 hours of cisplatin exposure (lanes 1-4). Figure 2B shows the topoisomerase I activity of SBC-3 cells. Supercoiled DNA bands appeared at one hour after cisplatin exposure (lane 7), which suggests a rapid decrease in topoisomerase I activity. Relaxed DNA appeared at 5 hours after cisplatin exposure (lane 3). Thus, in both cell lines topoisomerase I activity began to decrease at 1-2 hours after cisplatin exposure and recovered gradually after 4-5 hours of cisplatin exposure.

Discussion

We found that pretreatment with cisplatin augmented the sensitivity to the combination of SN-38 and cisplatin in ABC-1 and SBC-3 cells. Pretreatment with SN-38 also enhanced sensitivity in SBC-3 cells, but did not in ABC-1 cells. Accordingly, pretreatment with cisplatin might have a great impact on the tumor cell sensitivity to irinotecan and cisplatin.

In general, tumor cells with high topoisomerase I activity are sensitive to topoisomerase I inhibitors (20, 21). Thus, we expected the increase of topoisomerase I activity after cisplatin exposure. Surprisingly, the topoisomerase I activity of both cell lines began to decrease at 1-2 hours after cisplatin exposure, but gradually recovered after 4-5 hours of cisplatin exposure. Topoisomerase I activity was down-regulated and recovered during the time course after cisplatin exposure. We presume that the topoisomerase I enzyme is transiently consumed by its binding to DNA intercalated or intracalated by cisplatin. The consumption of topoisomerase I might contribute to the synergy when cisplatin is given first. These changes of topoisomerase I activity are also observed after irradiation exposure. Boothman *et al.* (21) reported that topoisomerase I inhibitors augmented the radiation effect, although topoisomerase I activity diminished immediately after irradiation.

The relationship between cisplatin and topoisomerase I activity is very complex. Although topoisomerase I activity or mRNA was higher in four cisplatin-resistant cell lines than their respective parent cell lines, two cell lines showed collateral sensitivity to topoisomerase I inhibitors (22, 23), which the other two cell lines did not (23, 24). In addition, two cisplatin-resistant cell lines showed cross-resistance to SN-38 without an alteration of topoisomerase I activity (25, 26). The relationship between the effect of cisplatin on topoisomerase I activity and the sensitivity to topoisomerase

I inhibitors is still unclear. The sensitivity to topoisomerase I inhibitors might be controlled by multifactors such as single-mutations in the topoisomerase I enzyme (27), topoisomerase I activity (19, 20), P-glycoprotein-associated multidrug resistance (28, 29) and intercellular drug accumulation (26, 30). Accordingly, we could not explain the mechanisms of synergy in terms only of topoisomerase I activity.

A synergistic effect on tumor cells *in vitro* might induce serious adverse reactions *in vivo*. In Japan, combination chemotherapy with cisplatin and irinotecan is the standard chemotherapy for NSCLC and extensive-stage SCLC (31, 32). de Jonge *et al.* have reported that the toxicity of the combination of irinotecan and cisplatin is schedule-independent (33); we expected a stronger synergistic effect in tumor cells than that in normal cells and conducted a new regimen of cisplatin and docetaxel on day 1 and irinotecan on day 2 (34).

In conclusion, pretreatment with cisplatin indicated the sensitivity of cancer cells to SN-38, a metabolite of irinotecan. This is a promising effect which should be further investigated *in vivo*.

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