A New Antimitotic Substance, FR182877
II. The Mechanism of Action

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Although antitumor drugs such as DNA synthesis inhibitors and antimitotic agents (microtubule modulators) have played important roles in the cancer chemotherapy, their clinical use is limited by their side effects. Thus, the continuing screening efforts have been made to discover more effective antitumor drugs.

In the previous paper[1], we have reported that a novel antimitotic substance, FR182877 isolated from the fermentation broth of *Streptomyces* sp. No. 9885, was discovered by developing a new screening method for antimitotic substances (Fig. 1).

In this note, we describe the effect of FR182877 on the cell cycle transition and the microtubule assembly *in vitro* as the mechanism of action of FR182877.

The phase studies of FR182877 on the cell cycle distribution of HT-29 cells were performed. The cells were treated with various concentration of FR182877 or known antitumor drugs and DNA contents were analyzed by flow cytometry. FR182877 and taxol[2,3] induced G_2/M phase arrest (Fig. 2). In addition, other antimitotic drugs such as vincristine[4] and rhizoxin[5] also induced G_2/M phase arrest, while inhibitors of DNA synthesis such as adriamycin and camptothecin induced S phase arrest (data not shown). A similar effect on cell cycle distribution was observed in human mammary adenocarcinoma cell M-8 cells[6]. As shown in Fig. 2, in HT-29 cells treated with 30 ng/ml of FR182877, the main population (57.5%) of cells was detected in G_2/M phase. FR182877 induced G_2/M phase arrest in the cell cycle. The concentration of FR182877 to induce G_2/M phase arrest was about three times higher than that of taxol. IC_{50} of FR182877 and taxol against HT-29 cells were 73 ng/ml and 12 ng/ml, respectively. The difference of concentration to induce cell cycle arrest may be reflected on the difference of IC_{50}.

Next, we examined the effect of FR182877 on the microtubule assembly by the turbidimetric assay. Tubulin was polymerized in the presence of 1 mM GTP and varying concentration of FR182877. From the results shown in Fig. 3, the rate of tubulin polymerization was influenced by FR182877 concentration and the time required for half-maximum microtubule assembly at each FR182877 concentration was also different. In addition, the polymerization curves of tubulin incubated with FR182877 were preceded by a lag phase. The time lag period was remarkably reduced at higher concentrations of FR182877 tested. Further, taxol induced the assembly of tubulin, while the anti-tubulin drugs such as vincristine and rhizoxin inhibited microtubule assembly (Fig. 3). These changes are consistent with the mechanism of action of the drugs[2,7].

The efficacy of FR182877 in the microtubule assembly was almost similar to that of taxol. However, the concentration of FR182877 to induce the microtubule assembly was about five to ten times higher than that of taxol. The kinetic analysis for microtubule assembly suggested involvement of more than one step of microtubule assembly; for example, a nucleation step followed by elongation or growth of microtubules. Detail of the kinetic studies of FR182877 is now in progress.

These results indicate that FR182877 promotes the microtubule assembly *in vitro* and induced G_2/M phase arrest in the cell cycle. This mode of action represents that FR182877 is a new structural class of microtubule-stabilizing agents such as taxol and epothilones[8]. In addition, FR182877 prolonged the life span of murine

Fig. 1. Chemical structure of FR182877.

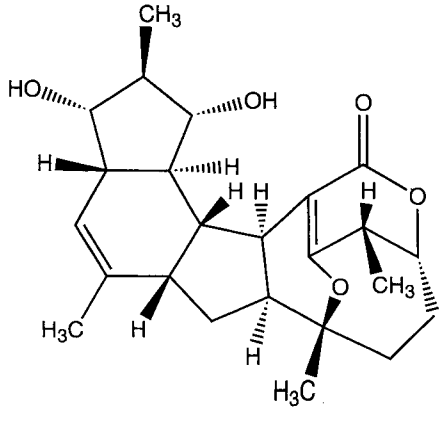

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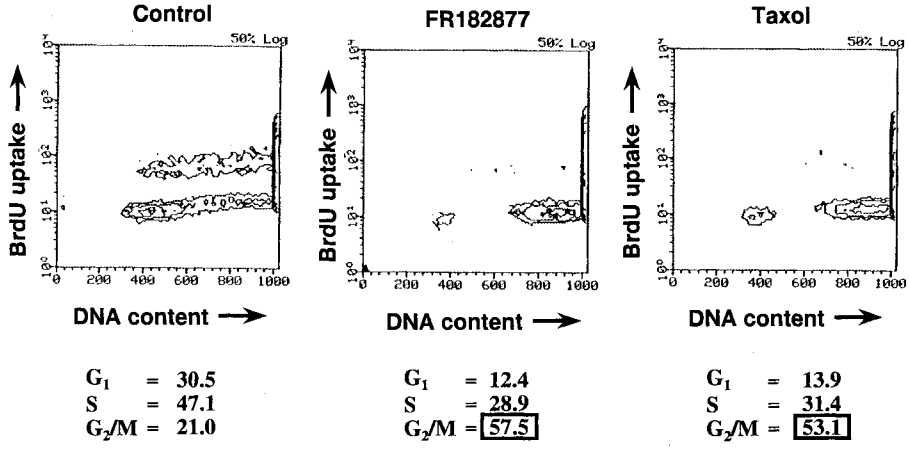
Fig. 2. Cell cycle analysis of HT-29 cells treated with FR182877 and taxol.



HT-29 cells were incubated for 16 hours in the presence or absence of FR182877 or taxol. After pulse-labeled with BrdU, cells were fixed, stained with a fluorescein-conjugated anti-BrdU antibody and PI, and analyzed by FACScan with LYSYS II software. Histograms show relative DNA content (abscissa) and level of DNA synthesis (ordinate). The percentage of cells in each phase of the cell cycle was quantified and shown under the histogram.

ascitic tumor P388 and inhibited the growth of murine solid tumor Colon 38[1]. It is suggested that FR182877 will be a new type of lead compounds for antitumor drugs.

Experiments

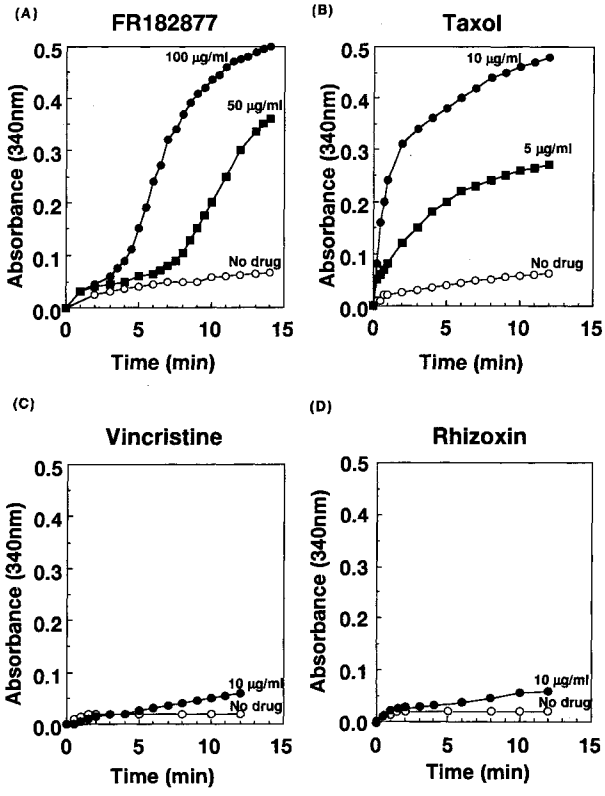
Cell Cycle Analysis

Human colon adenocarcinoma HT-29 cells were plated at 1×10^6 cells per 100-mm dish in the culture medium. Twenty-four hours later, test compound was added at various concentrations and incubated for 16 hours. 5-Bromo-2′-deoxyuridine (BrdU, 30 μg/ml) was incorporated into the DNA of the cells for 30 minutes to measure the rate of DNA synthesis. After pulse-labeled with BrdU, cells were collected by trypsinization, fixed and incubated with a fluorescein-conjugated anti-BrdU antibody. The nuclei were stained with propidium iodide (PI, 10 μg/ml) to measure DNA content per nucleus. Cell cycle phase distribution was analyzed by FACScan with LYSYS II software (Becton Dickinson).

Assay of Microtubule Assembly

Assembly of tubulin at 35°C was monitored by recording the change in turbidity at 340 nm using Hitachi 220A (UV-Visible) spectrometer. The temperature of the cuvette holder was maintained electronically and recorded at each time point of measurement. The cuvette (1 cm path)

Fig. 3. Polymerization curves of tubulin incubated with FR182877 or other antimitotic drugs.



Polymerization of tubulin at 35°C was monitored by recording the increase in absorbance at 340 nm in the presence of FR182877 (A), taxol (B), vincristine (C) or rhizoxin (D).

containing PEM buffer (80 mM piperazine-*N*,*N*'-bis(2-ethanesulfonic acid) sesquisodium salt, 1 mM magnesium chloride, 1 mM ethylene glycol-bis(6-amino-ethyl ether) *N*,*N*,*N*',*N*'-tetraacetic acid, adjusted to pH 6.8 with NaOH), 1 mM GTP and test compound were kept at room temperature before addition of tubulin (1 mg/ml at final concentration) and shifted to 35°C. FR182877 and taxol were dissolved in DMSO and stored at −20°C. The final concentration of DMSO (0.5%) had no detectable effect on the microtubule assembly.

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