NEW ANTITUMOR ANTIBIOTIC, FR-900405

III. MECHANISM OF ACTION OF FR-900405

SUMIO KIYOTO, TOSHIHIRO SHIBATA, YOSHIO KAWAI, YASUHIRO HORI, OSAMU NAKAYAMA, HIROSHI TERANO, MASANOBU KOHSAKA, HATSUO AOKI and HIROSHI IMANAKA†

Exploratory Research Laboratories, †Planning and Development Group, Fujisawa Pharmaceutical Co., Ltd. 2-1-6 Kashima, Yodogawa-ku, Osaka, 532 Japan

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FR-900405, isolated from the culture broth of *Actinomadura pulveracea* sp. nov. No. 6049, is a new antitumor antibiotic which contained sulfur in the molecule and represented a novel class of antitumor agents. It is highly active in mice against experimental tumors[1,2].

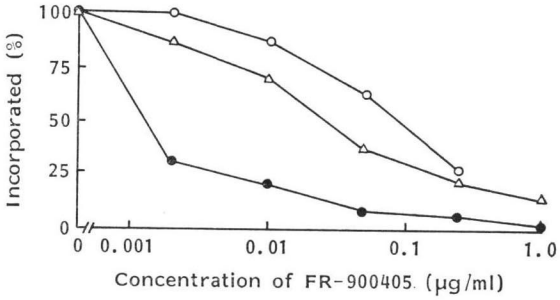
The mode of action was examined, and the results are described in this report.

Effect of FR-900405 on Macromolecular Synthesis in the Intact Cells of Mouse Leukemia L1210

Mouse leukemia L1210 cells were grown in DULBECCO's modified EAGLE's medium containing 10% fetal bovine serum in tissue culture flasks.

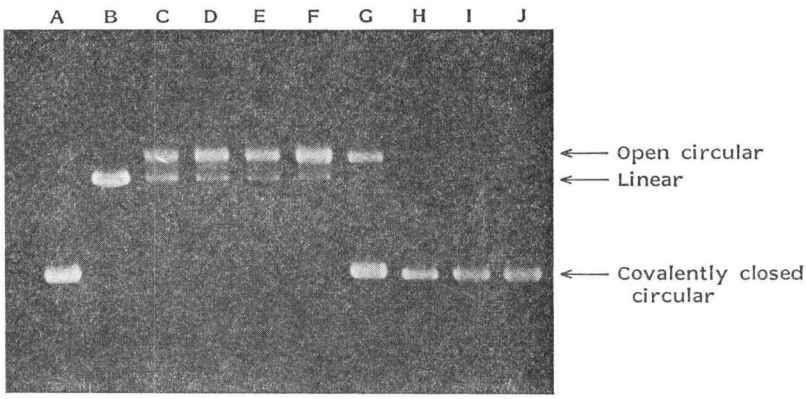
Fig. 1. Inhibition of macromolecular synthesis by FR-900405.

○ [^{3}H]Leucine, △ [^{3}H]uridine, ● [^{3}H]thymidine.



The initial cell number was 2.5×10^5 cells/ml. For investigations on macromolecular synthesis, 5 ml of cell suspension was incubated with various amounts of FR-900405 for 2 days, prior to the addition of 1 μCi/ml of [5,6-^{3}H]uridine, 2.5 μCi/ml of [*methyl*-^{3}H]thymidine, or 1 μCi/ml of L-[4,5-^{3}H(N)]leucine, respectively. The radiolabeled compounds were incorporated for 120 minutes at 37°C in 5% CO_2 incubator and duplicate 0.2 ml samples were taken from the culture. The incorporation was terminated by adding 10% trichloroacetic acid (TCA), and then the sample were filtered on a Millipore filter (pore size: 0.22 μm) for radioactivity count. Incorporation into TCA insoluble fraction of ^{3}H-labeled uridine, ^{3}H-labeled thymidine or ^{3}H-labeled leucine was observed as a measure of the synthesis of RNA, DNA and protein, respectively.

Fig. 2. Interaction of FR-900405 with pBR322 DNA.



A: Drug free control, B: *Pst* I-digested DNA (indicates the position of linear form DNA), C: FR-900405; 1,000 μg/ml (final concentration), D: FR-900405; 500 μg/ml, E: FR-900405; 100 μg/ml, F: FR-900405; 50 μg/ml, G: FR-900405; 10 μg/ml, H: FR-900405; 5 μg/ml, I: FR-900405; 1 μg/ml, J: Drug free control.

By the method employed, 50% inhibition of RNA, DNA and protein synthesis was observed at the FR-900405 concentration of 0.028 μg/ml, 0.0013 μg/ml and 0.1 μg/ml, respectively (Fig. 1). The results indicated that FR-900405 produced a preferential inhibition of DNA synthesis. A concentration required for a 50% inhibition of cell growth was approximately 0.005 μg/ml.

Interaction of FR-900405 with pBR322 DNA

In this section, interaction of FR-900405 with DNA was examined further in order to find how the antitumor antibiotic affects DNA molecules. pBR322 covalently closed circular (ccc) DNA was purchased from Takara Shuzo Co. Ltd.

The reaction mixture (20 μl) contained 0.8 μg DNA and various amounts of FR-900405 in 50 mM Tris-HCl buffer (pH 8.1). In another case, the reaction mixture contained 10 mM $MgCl_2$, 50 mM $(NH_4)_2SO_4$, 0.8 μg pBR322 DNA and restriction enzyme *Pst* I (Takara Shuzo Co. Ltd.) in 20 mM Tris-HCl (pH 7.5) to obtain the limit product of circular DNA (linear form DNA). Reaction was carried out for 1 hour at 37°C and stopped by addition of 5 μl of EDTA solution containing 50% (w/v) glycerol and 0.1% bromophenyl blue. The sample (25 μl) was directly analyzed by agarose gel electrophoresis as described by UEDA *et al.*[3].

This result showed in Fig. 2. FR-900405 caused both the single strand scission(s) and the double strand scission in pBR322 DNA. However, the precise mechanism of induction of DNA strand scission remains to be determined.

From these results, it suggested a direct interaction of FR-900405 with DNA.

References

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