New Antitumor Substances, FR901463, FR901464 and FR901465

II. Activities against Experimental Tumors in Mice and Mechanism of Action

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FR901463, FR901464 and FR901465, novel antitumor substances, were isolated from the fermentation broth of *Pseudomonas* sp. No. 2663. Their antitumor activities were examined in three mouse tumor systems and one human tumor system. The three FR compounds prolonged the life of mice bearing murine ascitic tumor P388 leukemia (T/C values were 160%, 145% and 127% for FR901463, FR901464 and FR901465, respectively), and inhibited the growth of a human solid tumor, A549 lung adenocarcinoma, with different effective dose ranges. FR901464 exhibited most prominent effects on these tumor systems among the three FR compounds. FR901464 also inhibited the growth of murine solid tumors, Colon 38 carcinoma and Meth A fibrosarcoma. To address the involvement of transcriptional activation ability of the three FR compounds in the antitumor effect, we selected FR901464 as a candidate compound and investigated cell cycle transition, chromatin status and endogenous gene expression in FR901464-treated tumor cells having elevated transcriptional activity. FR901464 induced characteristic G_1 and G_2/M phase arrest in the cell cycle and internucleosomal degradation of genomic DNA with the same kinetics as activation of SV40 promoter-dependent cellular transcription in M-8 tumor cells. In contrast to the potent activation of the viral promoter, FR901464 suppressed the transcription of some inducible endogenous genes but not house keeping genes in M-8 cells. These results suggest that FR901464 may induce a dynamic change of chromatin structure, giving rise to strong antitumor activity, and therefore may represent a new type of drug for cancer chemotherapy.

Although antitumor drugs have played important roles in cancer therapy, the known antitumor drugs such as DNA synthesis inhibitors or microtuble modulators are poorly active against solid tumors in the clinic. Thus, in addition to conventional screening efforts, new approaches to the treatment of cancer have been made with the hope of developing an antitumor drug with a different mode of action.

Recent progress in cancer research is noteworthy, resulting in an understanding of many steps of oncogenesis at the molecular level. The transcriptional regulation of genes is the final event conferred by various growth signals and controlled by the modification of chromatin structure and activated transcription factors. The important events in tumor cell growth such as cell cycle transition, production of growth factors or cell death are primarily controlled by transcriptional regulation[1,2]. Thus, the modulation of transcription is expected to become a new target to develop useful drugs against tumor cells which have impaired transcriptional regulation systems consisting of nuclear oncogene and tumor suppressor gene products.

We found that *Pseudomonas* sp. No. 2663 produced novel antitumor substances, FR901463, FR901464 and FR901465, which possess unique activation ability on SV40 promoter-dependent cellular transcription and potent cytotoxic effect against various human tumor cell lines[3,4].

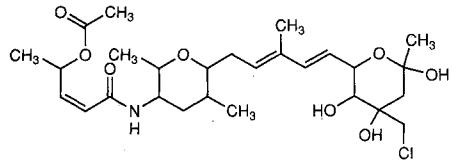
In this report, we describe the antitumor effects of FR901463, FR901464 and FR901465 against various murine and human standard tumors implanted in mice, and possible involvement of transcriptional regulation as the mechanism of FR901464.

Materials and Methods

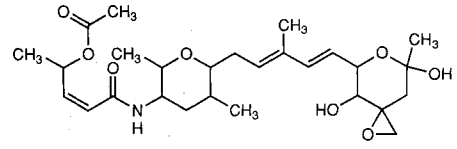
Drugs

FR901463, FR901464 and FR901465 were prepared in our Research Laboratories; their chemical structure are shown in Fig. 1. ADR was purchased from Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan. Camptothecin was purchased from Sigma chemical Co. Taxol was purchased from WAKO Pure Chemical Industries, Ltd.,

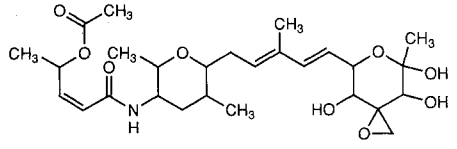
Fig. 1. Chemical structures of FR901463, FR901464 and FR901465.



FR901463



FR901464



FR901465

Osaka, Japan. FR901463, FR901464 and FR901465 were dissolved in and diluted with 10% polyoxyethylated (60 mol) hydrogenated castor oil in saline (HCO60 solution). The other drugs were dissolved in and diluted with saline. The solutions were given ip or iv to mice at a volume of 10 ml/kg body weight. In the *in vitro* culture test, all the drugs were dissolved in methanol and diluted with the culture medium described below.

Animals

Female mice of BALB/c, BDF_1 (C57BL/6 × DBA/2), DBA/2 and C57BL/6 strains were purchased from Charles River Japan Inc., Atsugi, Japan. Female mice of BALB/c nu/nu strain were purchased from CLEA Japan Inc., Tokyo, Japan.

Tumors

All tumor cell lines used were kindly gifted from Cancer Chemotherapy Center, Japanese Foundation for Cancer Reasearch. P388 leukemia (P388) cells were maintained ip by serial passage in DBA/2 mice. Colon 38 carcinoma (Colon 38) cells were maintained sc by serial passage in C57BL/6 mice. Meth A fibrosarcoma cells were maintained ip by serial passage in BALB/c mice. A549 lung adenocarcinoma (A549) cells were maintained sc by serial passage in BALB/c nu/nu mice.

Transformants

Human mammary adenocarcinoma MCF-7 cells were stably transformed with SV40 promoter-driven CAT reporter gene (pSV2-CAT) as described previously[3]. One of the clones obtained, named M-8 which expresses high levels of CAT protein constitutively, was used in the following experiments. M-8 cells were maintained and treated in the culture medium, DULBECCO's modified EAGLE's medium (DMEM) (Flow Laboratories, North Ryde, Australia) supplemented with 10% FBS (HyClone Laboratories, Logan, UT), penicillin (50 units/ml) - streptomycin (50 μg/ml) (Flow Laboratories), 4 μg/ml insulin (Sigma) and 10 nM estradiol (Sigma). The cells were grown and incubated in 5% CO_2-95% air atmosphere at 37°C.

Evaluation of Antitumor Effects on Murine Ascitic Tumors

P388 cells (1×10^6) were inoculated ip in BDF_1 mice. Five mice were used in the control and drug treated groups. Drug efficacy was assessed as a percentage of median survival time of the treated group (T) to that of the control group (C).

$$\text{T/C (\%)} = \frac{\text{Median survival time of (T)}}{\text{Median survival time of (C)}} \times 100$$

Evaluation of Antitumor Effects on Human and Murine Solid Tumors

In the experiments on murine solid tumors, fragments ($2 \times 2 \times 2$ mm) of Colon 38 were implanted sc in the left flank of BDF_1 mice. Meth A cells (1×10^5) were inoculated intradermally (id) in BALB/c mice. In both experiments, 10 mice were used per group.

Tumor weight, as derived from caliper measurements of the length (a) and width (b) of tumors in mm, was calculated by the equation: tumor weight (mg) $= 1/2 \times a \times b^2$. Drug efficacy against murine solid tumors was based on the percentage of mean tumor weight of the treated group (T) to that of the control group (C).

$$\text{Growth inhibition (\%)} = \left(1 - \frac{\text{Mean tumor weight (T)}}{\text{Mean tumor weight (C)}}\right) \times 100$$

In the experiment on human solid tumor A549, a 2-week subrenal capsule (SRC) assay by using the immunosuppressive agent FK-506 which was developed in our Research Laboratories was employed[5,6]. The method of implanting tumors under kidney capsule of the mouse was described by BOGDEN *et al.*[7,8]. A 1-mm^3 tumor fragments were implanted under kidney capsule of BDF_1 mice on Day 0 and on Day 14 mice were sacrificed, the tumor bearing kidney was exteriorized and the final size of the implanted tumor was measured as described above. FK-506 (32 mg/kg) was injected sc on Day 1, 2, 5, 7, 9 and 12 which completely inhibited the immune response for rejecting the xenograft.

Drug efficacy was calculated by the same equation as murine solid tumors (as described above).

Activity Criteria

The criteria for activity and toxicity in *in vivo* tumor model were estimated according to a modification of the method used by the National Cancer Institute[9]. We used two criteria as described in each table, and defined the

small and large values in T/C or 1-T/C (%) as moderate (+) and good activities (++), respectively.

Quantification of CAT Proteins

M-8 cells (1×10^5) cultured in 24 well multiwell plate were incubated for the indicated times in the culture medium containing 10 ng/ml of FR901464. CAT induction was terminated by replacing the medium with 100 μl of 0.25 M Tris-HCl, pH 7.8. After three cycles of freezing and thawing, cell lysates were collected and CAT protein level was determined by the colorimetric enzyme immunoassay using CAT ELISA (Boehringer Mannheim GmbH) according to the manufacturer's instructions.

Cell Cycle Analyses

M-8 cells were plated at 1×10^6 cells per 100-mm dish in the culture medium. Twenty four hours later, FR901464 or other drugs was added to cultures at various concentrations and incubated for 16 hours. In the experiment on kinetics, 10 ng/ml of FR901464 was added and incubated for the indicated times. In the experiment to study the effect of FR901464 on cell cycle transition after release from serum starvation, M-8 cells were starved in serum-free medium for three days. Cells refed with 10% of serum were treated with 10 ng/ml of FR901464 for various times. In each experiment, methanol was added to the control culture. 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. Cells were collected by trypsinization, fixed, and the isolated nuclei were incubated with a fluorescein-conjugated antibody (Becton Dickinson) that binds to BrdU in the DNA. The nuclei were also 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).

Cell Death Analysis

M-8 cells were seeded as described above and incubated in the presence or absence of 10 ng/ml of FR901464 for the various times. Cells were collected, lyzed and the enrichment of mono- and oligonucleosomes in cytoplasm of treated cells was measured by the photometric enzyme immunoassay using Cell Death Detection ELISA (Boehringer Mannheim GmbH) according to the manufacturer's instructions.

Analyses of Endogenous Gene Expression

M-8 cells were seeded as described above and treated with or without 10 ng/ml of FR901464 for 16 hours. Cells were collected by the cell scraper and total RNA was purified by RNeasy Total RNA kit (QIAGEN GmbH) according to the manufacturer's instructions. RNA concentrations were determined by using GeneQuant RNA/DNA Calculator (Pharmacia Biotech). Equal amounts of RNA of each sample were subjected to RT-PCR amplification using RNA PCR Kit (TaKaRa) and DNA Thermal Cycler 480 (PERKIN ELMER) according to the manufacturer's instructions. Primers used for cDNA amplification of endogenous genes were as follows:

primers for CAT
5'-primer 5'-CAATGTACCTATAACCAGACCG-3'
3'-primer 5'-GCATGATGAACCTGAATCGCCAG-3'
primers for human c-myc
5'-primer 5'-TACCCTCTCAACGACAGCAGCTCGCCC-AACTCCT-3'
3'-primer 5'-TCTTGACATTCTCCTCGGTGTCCGAGG-ACCT-3'
primers for human E2F-1
5'-primer 5'-CGCTATGAGACCTCACTGAATCTG-3'
3'-primer 5'-GCGTAGTACAGATATTCATCAGG-3'
primers for human p53
5'-primer 5'-CTGAGGTTGGCTCTGACTGTACCACCA-TCC-3'
3'-primer 5'-CTCATTCAGCTCTCGGAACATCTCGAA-GCG-3'
primers for human cip1[1)]
5'-primer 5'-AAGCTTGGATCCTCAGAGGAGGCGCC-ATGTCAGAA-3'
3'-primer 5'-AAGCTTGGATCCTTCCTGTGGGCGGAT-TAGGGCTTCCTC-3'
primers for human β-actin
5'-primer 5'-ATCTGGCACCACACCTTCTACAATGAG-CTGCG-3'
3'-primer 5'-CGTCATACTCCTGCTTGCTGATCCACA-TCTGC-3'
primers for human G3PDH
5'-primer 5'-TGAAGGTCGGAGTCAACGGATTTGGT-3'
3'-primer 5'-CATGTGGGCCATGAGGTCCACCAC-3'

Primers for c-myc, p53, β-actin and G3PDH were purchased from CLONTECH Laboratories, Inc. Amplification of cDNA was carried out for 25 cycles (following the profile of 94°C for 1 minute, 60°C for 1 minute, 72°C for 2 minute), yielding a 494 bp, 479 bp, 307 bp, 371 bp, 523 bp, 838 bp and 983 bp PCR product for CAT, c-myc, E2F, p53, cip-1, β-actin and G3PDH, respectively. After amplification, amplified DNA was electrophoresed on 2% agarose gel and stained with ethidium bromide for analysis.

Results

Antitumor Activity against Mouse Ascitic Tumors

The antitumor activities of FR901463, FR901464 and FR901465 against mouse ascitic P388 were examined. The tumor cells were inoculated ip in mice on Day 0. The drugs were given ip to mice once a day for 4 days (Days 1~4). As shown in Table 1, the three FR compounds prolonged the life span of tumor bearing mice, although FR901465 was less active than FR901463 and FR901464. The effective dose ranges of FR901463 and FR901464 are 1.0 to 3.2 mg/kg and 0.056 to 0.18 mg/kg, respectively.

Table 1. Antitumor effects of FR901463, FR901464 and FR901465 on murine ascitic tumors in mice.

Dose (mg/kg)	P388 (ip-ip) QD[a] D1~4					
	FR901463		FR901464		FR901465	
	T/C (%)	Activity[b]	T/C (%)	Activity	T/C (%)	Activity
0.0032					109	-
0.01					118	-
0.018			118	-		
0.032			109	-	127	+
0.056			136	+	27	Tox
0.1	100	-	145	+		
0.18			136	+		
0.32	110	-	55	Tox		
1.0	160	+				
3.2	140	+				
10	22	Tox[c]				

[a] QD, every day.
[b] Tumor cells were inoculated ip to mice on Day 0. The drugs were given ip to mice once a day for 4 days (days 1~4). Criteria: +, ≧120 and ++, ≧175.
[c] Tox, a T/C value of <86% indicates toxicity.

Table 2. Antitumor effects of FR901463 and FR901464 on human solid tumor in mice.

Dose (mg/kg)	A549 (SRC-ip) Q4D[a] Days 1, 5 and 9 weight D14			
	FR901463		FR901464	
	1-T/C (%)	Activity[b]	1-T/C (%)	Activity
0.056			36	-
0.1			61	+
0.18			63	+
0.32			77	+
0.56	5	-	83	+
1.0	-6	-		Tox
1.8	13	-		
3.2	61	+		
5.6		Tox[c]		

[a] Q4D, every 4 day.
[b] Tumor cells were implanted under kidney capsule of the mouse on Day 0. The drugs were given ip to mice three times at 4-days intervals beginning on Day 1. Tumor weights were measured on day 14. Criteria: +, ≧58 and ++, ≧90.
[c] Tox, a survival rate of <65% on evaluation day indicates toxicity.

Antitumor Effect on Human Solid Tumor in SRC Assay

The antitumor effects of FR901463 and FR901464 were examined in the SRC assay against human lung adenocarcinoma A549. The 2-week SRC assay by using the immunosuppressive agent FK-506 was employed. A549 was implanted at Day 0 under kidney capsule of the mouse. The drug was given ip 3 times at 4-days intervals, and the tumors were weighed on exteriorized kidneys. FK-506 was injected sc to inhibit the immune response rejecting the xenograft. As shown in Table 2, FR901464 exhibited a prominent antitumor effect against A549. The effective dose range showed 0.1 to 0.56 mg/kg. On the other hand, FR901463 showed a weaker antitumor effect.

Antitumor Effects on Murine Solid Tumors in Mice

The antitumor effects of FR901464 were examined on murine solid tumors in mice. Colon 38 and Meth A were implanted sc and id on Day 0, respectively. The drug was given iv once a day on 3 or 4 nonconsecutive days, and the tumors were weighed. The results are shown in Table 3. FR901464 inhibited the growth of Colon 38 and Meth A in the dose dependent manner with the dose range from 0.18 to 1 mg/kg.

Table 3. Antitumor effects of FR901464 on murine solid tumors in mice.

Drug	Dose (mg/kg)	Colon 38 (sc-iv) Q3D[a] Days 1, 4, 7 and 10 weight D21		Meth A (id-iv) Q3D Days 8, 11 and 14 weight D21	
		1-T/C (%)	Activity[b]	1-T/C (%)	Activity
FR901464	0.1	-13	-	21	-
	0.18	0	-	43	-
	0.32	46	-	38	-
	0.56	68	+	46	-
	1.0		Tox[c]	86	+

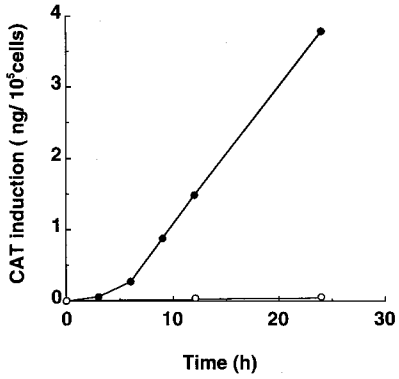
[a] Q3D, every 3 day.
[b] Tumor cells were implanted sc or id to mice on Day 0. In the Colon 38 test, the drugs were given iv to mice once a day on Days 1, 4, 7 and 10. In the Meth A test, the drugs were given iv to mice once a day on Days 8, 11 and 14. Tumor weights were measured on Day 21. Criteria : +, ≧58 and ++, ≧90.
[c] Tox, a survival rate of <65% on evaluation day indicates toxicity.

Kinetics of Transcriptional Activation Induced by FR901464

M-8 cells, which harbored the SV40 promoter-driven CAT reporter gene, were employed to measure the activities on cellular transcription. FR901464 has a profound enhancing effect on cellular transcription in M-8 cells[3]. To address whether transcriptional activa-

Fig. 2. Kinetics of CAT expression induced by FR901464 in M-8 cells.

● FR901464 (10 ng/ml), ○ control.



M-8 cells were incubated for varying periods of time in the presence (●) or absence (○) of FR901464. CAT protein was extracted by freezing and thawing of cells. Cell lysates were collected and CAT protein level was determined in duplicate by colorimetric enzyme immunoassay using CAT ELISA (Boehringer Mannheim GmbH). CAT protein in each sample was indicated as ng protein/10^5 cells, calculated by the calibration curve of the CAT enzyme standard.

tion induced by FR901464 is involved in its suppressive effect of tumor cell growth, the kinetics of CAT expression in FR901464-treated M-8 cells were investigated. CAT protein induced by FR901464 was quantified using a colorimetric enzyme immunoassay. As shown in Fig. 2, CAT protein was first detected at 6 hours post-treatment and continued increasing up to 24 hours in FR901464-treated cells, although it failed to be detected in control cells.

Effects of FR901464 on Cell Cycle Distribution of M-8 Cells

Phase and kinetic studies of FR901464 on cell cycle distribution of M-8 cells were performed. The BrdU-labeled nuclei from cells treated with FR901464 and other antitumor agents were isolated and incubated with a fluorescent antibody directed against BrdU. The nuclei were then stained with PI. A fluorescence-activated cell sorter (FACS) was employed to analyze DNA content evaluated with PI fluorescence and the level of BrdU incorporation to measure recent DNA synthesis. As shown in Fig. 3 A, in M-8 cells, treatment with known antitumor drugs such as inhibitors of DNA synthesis, adriamycin and camptothecin, induced S phase arrest in the cell cycle, while a microtuble modulator, taxol, induced G_2/M phase arrest. These changes in cell cycle distribution are consistent with the mechanism of action of the drugs.

On the other hand, in M-8 cell treated with FR901464, a significant decrease in cells entering S phase and exiting from G_2/M phase were observed (Fig. 3B and 3C). This result indicates that FR901464 induces G_1 and G_2/M phase arrest in the treated cells.

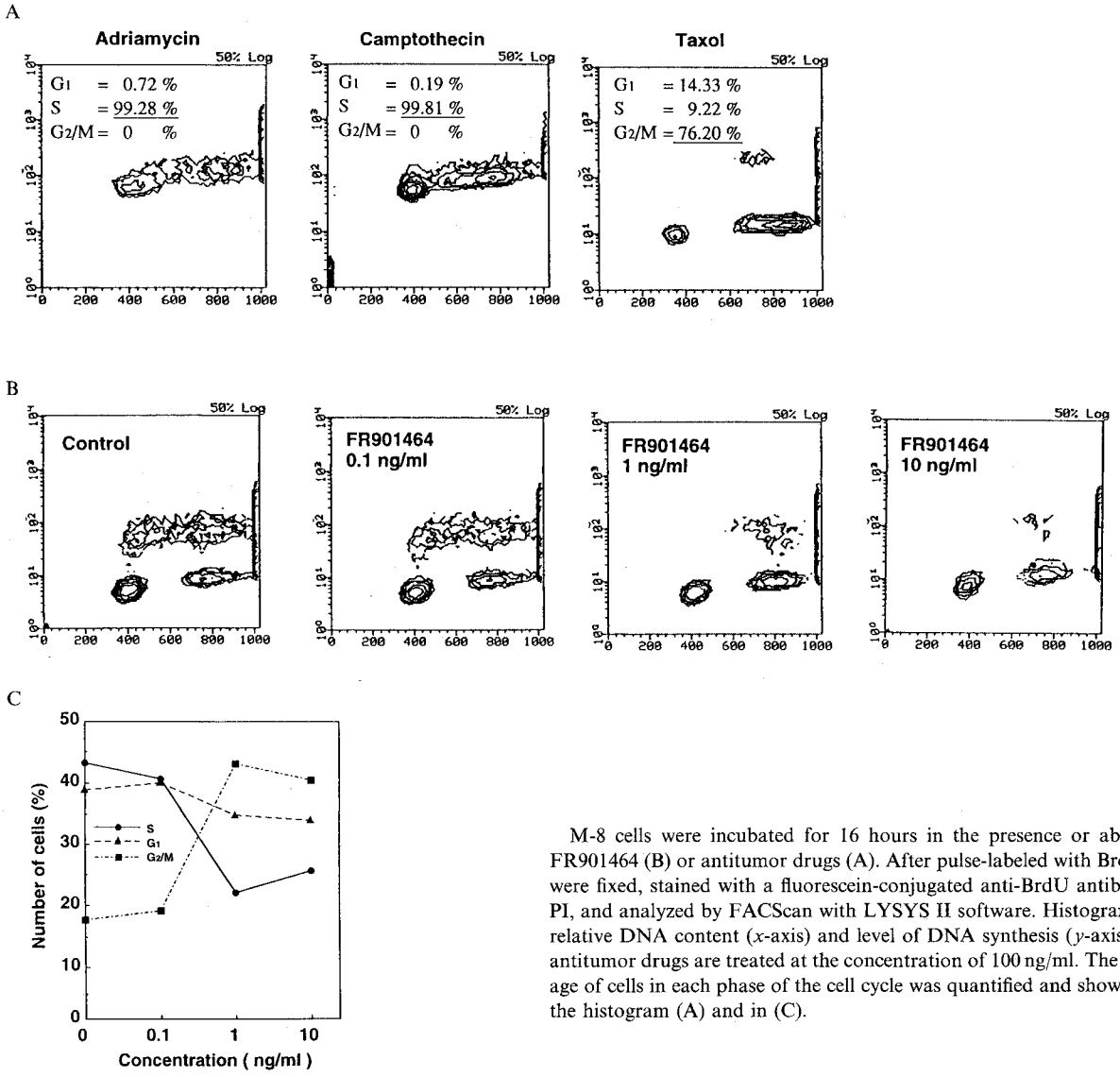
This was also defined by the effect of FR901464 on cell cycle transition of M-8 cells after release from serum starvation. As shown in Fig. 4, in starved M-8 cells, the main population of cells was detected in G_1 phase. The stimulation of starved cells with serum was followed by a change of cell cycle transition, which decreases G_1 phase cells and increases S phase cells. The number of cells in G_2/M phase remained constant. In contrast, in serum-refed M-8 cells treated with FR901464, marked decrease in S phase cells and simultaneous increase in G_2/M phase cells were observed, although the cell population of G_1 phase was unchanged. These results confirm the characteristic effect of FR901464 on cell cycle distribution to be G_1 and G_2/M phase arrest.

As shown in Fig. 5, kinetic study of cell cycle distribution in FR901464-treated M-8 cells verified that the changes of cell cycle distribution induced by FR901464 are first detected 6 hours after treatment and continue up to 24 hours, with increasing magnitude. A similar effect on cell cycle distribution was observed in human lung adenocarcinoma A549, human colon adenocarcinoma HT29, human fibrosarcoma HT1080 and mouse T cell lymphoma EL4 when treated with FR901464 (data not shown).

Analysis of Cell Death Induced by FR901464

FR901464 has a potent cytotoxic effect on M-8 cells, accompanied by a striking cell shrinkage which is frequently observed in cells with induced DNA fragmentation. To verify whether transcriptional activation induced by FR901464 is implicated in this characteristic cell death, chromatin status in the FR901464-treated cells was examined. Chromatin status was evaluated by quantifying the mono- and oligonucleosomes level in the cytoplasm using the photometric enzyme immunoassay. As expected, an enrichment of nucleosomes in the cytoplasm of FR901464-treated M-8 cells was observed, suggesting that internucleosomal breakdown of chromatin was induced (Fig. 6). Moreover, kinetic analysis verified that DNA fragmentation was occurring within 12 hours posttreatment and continued for up to 24 hours, with increasing intensity.

Fig. 3. Flow cytometric cell cycle analysis of M-8 cells treated with FR901464 and known antitumor drugs.



M-8 cells were incubated for 16 hours in the presence or absence of FR901464 (B) or antitumor drugs (A). 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 (x-axis) and level of DNA synthesis (y-axis). Three antitumor drugs are treated at the concentration of 100 ng/ml. The percentage of cells in each phase of the cell cycle was quantified and shown within the histogram (A) and in (C).

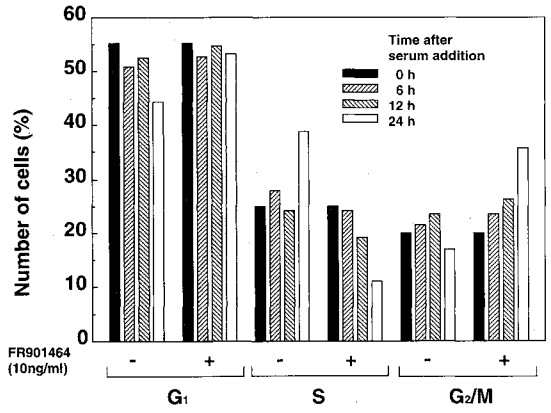
Effect of FR901464 on the Endogenous Gene Expression in M-8 Cells

FR901464 greatly enhanced the cellular transcriptional ability, monitored with the introduced SV40 promoter activity. Therefore, whether FR901464 also activates the transcription of endogenous genes as well as ectopic gene was investigated. Total RNA was extracted from the treated cells and changes in transcript levels of endogenous genes were compared with that of CAT gene, employing RT-PCR method. Human c-myc, E2F-1, p53 and p21 cip-1genes were selected and their expressions examined in M-8 cells as representative inducible genes because of their implication in cell cycle transition and cell death. β-actin and G3PDH genes were also employed as house keeping genes. As shown in Fig. 7, indeed, the expression of CAT gene was markedly enhanced in FR901464-treated M-8 cells. However, unexpectedly, the expression of all of the inducible genes examined was remarkably suppressed in the treated cells, whereas that of house keeping genes remained unchanged.

Discussion

FR901463, FR901464 and FR901465 were found through a newly developed *in vitro* screening system for transcriptional regulators[3]. They significantly enhanced SV40 promoter-dependent cellular transcription and potently inhibited the growth of murine and human tumor cells *in vitro*[3]. Regulation of transcription is expected to be an intriguing target to develop a new type

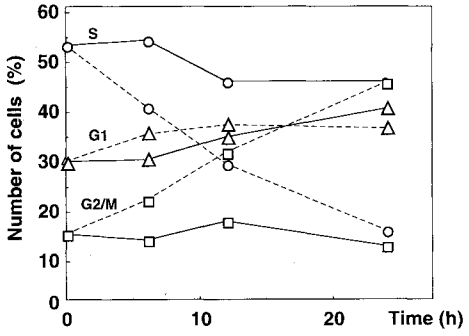
Fig. 4. Cell cycle distribution of M-8 cells after release from serum-starvation.



M-8 cells were serum-starved for 72 hours. Then, cells were refed by addition of serum at final concentration of 10% with or without 10 ng/ml of FR901464 for the times indicated. Cell cycle distribution was analyzed as described in Fig. 3 legend and the percentage of cells in each phase of the cell cycle was quantified.

Fig. 5. Kinetics of cell cycle distribution in M-8 cells treated with FR901464.

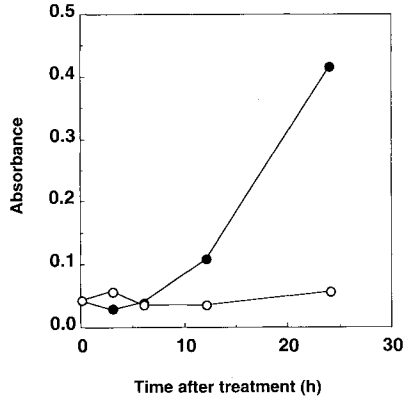
—— No drug, ----- FR901464 10 ng/ml. S: ○, G_1: △, G_2/M: □



M-8 cells were incubated in the presence (-----) or absence (——) of 10 ng/ml of FR901464 for the various times. Cell cycle distribution was analyzed as described in Fig. 3 legend and the percentage of cells in each phase of the cell cycle was quantified.

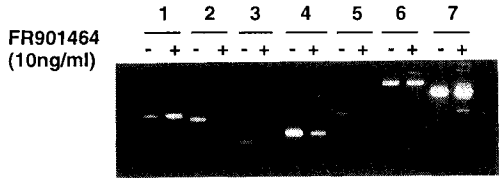
Fig. 6. Enrichment of nucleosomes in the cytoplasm of M-8 cells treated with FR901464.

● FR901464 10 ng/ml, ○ control.



M-8 cells were incubated for varying periods of time in the presence (●) or absence (○) of FR901464. Cells were lyzed, and mono- and oligonucleosome level were determined in duplicate by photometric enzyme immunoassay using Cell Death Detection ELISA (Boehringer Mannheim GmbH). Nucleosome level of each sample was indicated as the absorbance at 405 nm.

Fig. 7. Effect of FR901464 on the expression of endogenous genes in M-8 cells.



M-8 cell were treated with (+) or without (−) 10 ng/ml of FR901464 for 16 hours. Total RNA was extracted and subjected to RT-PCR amplification. Amplification of cDNA was carried out for 25 cycles. PCR products of 494 bp for CAT (lane 1), 479 bp for c-myc (lane 2), 307 bp for E2F-1 (lane 3), 371 bp for p53 (lane 4), 523 bp for p21 cip-1 (lane 5), 983 bp for G3PDH (lane 6) and 838 bp for β-actin (lane 7) were yielded when resolved in 2% agarose gel.

of antitumor drug. We therefore examined the antitumor activities of FR901463, FR901464 and FR901465 against several mouse and human tumor cells and investigated the mechanism of action of FR901464.

As shown in Tables 1 and 2, FR901463, FR901464 and FR901465 prolonged the life of mice bearing ascitic tumors. In addition, FR901463 and FR901464 exhibited antitumor effects in the SRC assay using human solid tumor A549. In these assay systems, FR901464 showed the most prominent effects among the three compounds. Thus, we decided to select FR901464 as a promising candidate compound, and investigated further its antitumor effect against the murine solid tumors and mechanism of action. As expected, FR901464 also inhibited the growth of the murine tumors, Colon 38 and Meth A (Table 3). FR901464 is now being examined for its antitumor effects by the National Cancer Institute, U.S.A.

The transcriptional regulation of genes is known to result from interactions between *cis*-acting DNA elements and *trans*-regulatory factors which bind DNA. These interactions can be modulated by a conformational change in chromatin structure[10~13]. The minimum subunit of chromatin is the nucleosome in which DNA is tightly wound around a histone octamer. In transcriptionally active chromatin, the packaged chromatin structure is partially disrupted by structural transition in the nucleosome or by displacement and repositioning of nucleosomes, resulted in increasing accessibility of transcription factors to nucleosomal DNA or showing the hyper sensitivity against nucleases which cleave linker regions connecting nucleosomes and

produce the fragmented DNA[12,14~15]. On the other hand, keeping the integrity of chromatin structure is the essential requirement in the cell cycle transition[16]. Therefore, cells containing mutated DNA or disordered chromatin structure fail to proceed the cell cycle beyond the G_1 or G_2/M phase in which the check systems of the genome function[17].

Thus, it was demonstrated above that FR901464 enhanced transcription, arrested the cell cycle transition in G_1 and G_2/M phases and induced internucleosomal DNA fragmentation, and also shown that these biological events occurred with almost the same kinetics, suggesting these are triggered by a common effect. In addition, FR901464 was verified to be a transcriptional suppressor rather than activator against inducible genes which may require a strict regulation of displacement and repositioning of nucleosomes for transcriptional activation.

Taken together, these results strongly suggest that the first action of FR901464 in treated cells may be to disrupt the chromatin structure by an unknown mechanism and produce "biologically active chromatins", leading to the transcriptional activation of the integrated virus promoter but suppression of endogenous inducible genes, internucleosomal DNA fragmentation digested by endogenous nucleases and cell cycle arrest at check points, as mentioned above. This is also supported by the fact that a part of FR901464 action can be replaced with that of trichostain A, which is a potent inhibitor for histone deacetylase[15,18~19].

Although further experiments are necessary to verify the target protein of FR901464, the effort will certainly contribute to the screening for new types of antitumor drugs.

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