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E7070, a novel sulphonamide agent with potent antitumour activity *in vitro* and *in vivo*

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Abstract

E7070 (*N*-(3-Chloro-7-indolyl)-1,4-benzenedisulphonamide) was selected from our sulphonamide compound collections via antitumour screening and flow cytometric analysis. Following treatment with E7070, the cell cycle progression of P388 murine leukaemia cells was disturbed in the G1 phase. The cell-killing effect on human colon cancer HCT116 cells was found to be time-dependent. In the panel of 42 human tumour cell lines, E7070 showed an antitumour spectrum that was distinct from those of other anticancer drugs used in clinic. Animal tests using human tumour xenograft models demonstrated that E7070 could cause not only tumour growth suppression, but also tumour regression in three of five colorectal and two of two lung cancers. In the HCT116 xenograft model, E7070 was shown to be superior to 5-FU, MMC and CPT-11 (irinotecan). Furthermore, complete regression of advanced LX-1 tumours was observed in 80% of E7070-treated mice. All of these observations have promoted this drug to clinical evaluation.

Keywords: E7070; Sulphonamide; G1 phase; Human tumour xenograft model

1. Introduction

Disordered proliferation is among the most distinctive features of malignant tumours. Cell growth and differentiation in normal cells are strictly regulated by precise molecular mechanisms that are associated with the cell cycle machinery. However, in cancer cells, however, several abnormalities have been found in these mechanisms. Critical mutations in oncogenes (e.g. *ras* and *myc*) and tumour suppressor genes (e.g. *TP53* and *RB*) have been reported to accelerate cell cycle progression inappropriately, leading to a malignant cancerous state. Therefore, cell cycle inhibitors that prevent chaotic tumour growth have been considered promising candidates for anticancer chemotherapy. Thus far, we have screened a number of synthetic compounds with respect to not only their antiproliferative activity, but also their effect on cell cycle progression. According to this procedure, a mitotic-arrest sulphonamide E7010 was discovered as a novel tubulin polymerisation inhibitor [1,2]. The compound was shown to be quite active against various rodent and human tumours *in vivo* [3], entering phase I clinical trials as an orally active antimitotic agent [4]. Interestingly, in the course of testing compounds structurally related to E7010, we also discovered another class of antitumour sulphonamides and following treatment with such agents P388 murine leukaemia cells accumulated in the G1 phase, but not in the M phase of the cell cycle [5,6]. Intensive screenings, particularly using flow cytometric analyses and human tumour xenograft models, have resulted in the selection of E7070 as an optimised drug candidate [7].

Recent advances in cancer biology have clarified that the G1 phase of the cell cycle is an important period where various complex signals interact to determine a cell's fate: proliferation, quiescence, differentiation or apoptosis [8,9]. It is now well-recognised that there are significant differences in the regulation of the G1 phase between normal and cancer cells [10]. Thus, there appears to be a growing possibility that a small molecule targeting the control machinery in G1 can be a new

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type of anticancer drug. In the current anticancer chemotherapy, drugs disrupting cell cycle progression in the S, G2 or M phase are widely used because of their well-established clinical efficacy. Although there are no drugs approved for clinical use that disturb the cell cycle in G1, several new drug candidates belonging to this class, such as UCN-01, AG1295, AGM-1470, 17-allylaminogeldanamycin and flavopiridol [11–15], are being evaluated in clinical trials. Herein we describe the preclinical antitumour profile of E7070 that was independently discovered as a novel G1-targeting agent.

2. Materials and methods

2.1. Antitumour agents

E7070 was synthesised at Eisai Co. Ltd. (Tsukuba Research Laboratories, Ibaraki, Japan). The chemical structure is shown in Fig. 1. Etoposide, camptothecin and cisplatin (CDDP) were purchased from Sigma (St. Louis, MO, USA). 5-Fluorouracil (5-FU) was from Tokyo Kasei (Tokyo, Japan). Bleomycin was obtained from Nihon Kayaku (Tokyo, Japan). Mitomycin C (MMC) and doxorubicin (ADM) were from Wako (Osaka, Japan). All drugs were dissolved in dimethylsulphoxide (DMSO) for *in vitro* studies.

For animal tests, we used 5-FU and MMC purchased from Kyowa Hakko Kogyo Co. Ltd. (Tokyo, Japan), and CPT-11 (Topotecin®) from Daiichi Pharmaceutical Co. Ltd. (Tokyo, Japan). E7070 was dissolved at appropriate concentrations in 3.5% DMSO and 6.5% Tween 80 in saline. 5-FU and CPT-11 were each diluted with saline at an appropriate concentration. MMC was dissolved with saline at an appropriate concentration. All drugs were administered intravenously (i.v.) at the volume of 0.2 ml/mouse.

2.2. Cell lines

P388, LX-1 and MX-1 were supplied by the Cancer Chemotherapy Center, Japan Foundation for Cancer Research (Tokyo, Japan). NCI-H460, NCI-H520, NCI-H596, HCT116, SW480, SW620, COLO205, DLD-1, HCT-15, HT-29, MIApaca2, 5637, SCaBER, PC-3, LNCaP.FGC, DU145, MDA-MB-468, A-427 and HL-60 were purchased from American Type Culture Collection (Manassas, VA, USA). PC1, PC9, MKN28 and MKN74 were from Immuno Biology Laboratory (Gumma, Japan). A375, CCRF-CEM, A549, WiDr, LS174T, COLO320DM, PANC-1, T24, ACHN, G-361, ZR-75-1 and K-562 were from Dainippon Pharmaceutical Co. Ltd. (Osaka, Japan). PC10, MKN45, KATOIII and HMV-1 were obtained from the Japanese Cancer Research Resources Bank (Tokyo, Japan). MOLT-4 was supplied from the Institute of Cancer Research, Hokkaido University (Hokkaido, Japan). All cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 media containing 10% heat-inactivated fetal bovine serum, penicillin (100 units/ml), streptomycin (100 μg/ml), 2-mercaptoethanol (50 μM), and sodium pyruvate (1 mM). Cultured cells were maintained at 37? in a humidified chamber containing 95% air and 5% CO_2.

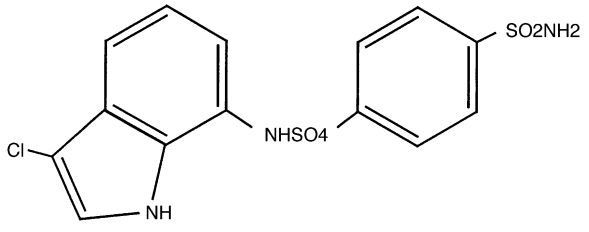

Fig. 1. Chemical structure of E7070 (*N*-(3-chloro-7-indolyl)-1,4-benzenedisulphonamide).

2.3. Flow cytometry

P388 cells were seeded at $1.25–10\times10^5$ cells/well in 24-well plates. After E7070 was added to each well, the cells were incubated for 12, 24 or 48 h. At the indicated time points, the cells were fixed in 70% ethanol at 4 °C for 1 h, stained with propidium iodide (50 μg/ml), and then analysed for DNA content by quantitation of red fluorescence in a flow cytometer (EPICS-CS).

2.4. In vitro *antitumour activity*

For the time course of E7070′s cytotoxicity, HCT116 cells were seeded at 10 000 cells/well in 96-well microtitre plates. After preincubation for 1 day, graded concentrations of test compounds were added to the plates (day 0). On days 0, 1, 2, 3 and 6, relative cell numbers were determined by the Sulphorodamine B (SRB) method [16].

For the *in vitro* antitumour spectrum, Exponentially growing cells were seeded at 1250–10 000 cell/well in 96-well microtitre plates. After preincubation for 1 day, graded concentrations of test compounds were added. Following the continuous incubation for additional 3 days, the IC_{50} value of each drug was determined based on the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay [17] by the least-squares method.

2.5. In vivo *human tumour xenograft models*

Female BALB/c *nu*/*nu* mice aged 7 weeks (SLC, Shizuoka, Japan) were housed in barrier facilities with food and water *ad libitum*. Cell suspensions of HCT116, SW620, HCT15, PC-9, DLD-1, WiDr or tumour fragments of LX-1 were implanted subcutaneously (s.c.) into the mice. When the tumour volume reached 100–200 mm^3 (day 1), i.v. administration was started. The

tumour volume (TV) was measured once or twice per week. On day 21 or 22, the tumours were removed and weighed. TV was calculated as: TV (mm^3) = length (mm) × width^2 (mm^2)/2. Relative tumour volume (RTV) was calculated as: $RTV = TV/TV_1$, where TV_1 is the tumour volume on day 1. The minimum value for RTV on average was designated as RTV_{min}. The T/C was the ratio of average tumour weight of the treated group to that of the control group. The relative body weight (RBW) was calculated as: $RBW = BW/BW_1$, where BW_1 is the body weight on day 1. The minimum value of RBW on average was designated as RBW_{min}. Antitumour effect was assessed based on the following two criteria; T/C for tumour growth suppression and RTV_{min} for tumour regression.

3. Results

3.1. Accumulation of P388 cells in the G1 phase following E7070 treatment

The effect of E7070 on the cell cycle progression of P388 murine leukaemia cells was examined at varying doses and time points by flow cytometry (Fig. 2). Following the 12-h drug treatment, the cells were accumulated in the G1 phase at 33 μg/ml and higher. By extending the drug exposure time, the G1 proportion was clearly increased even in the lower concentration range: 0.41–3.7 μg/ml for 24-h exposure and 0.14–1.2 μg/ml for the 48-h exposure, respectively. Dead cells and sub-G1 population were detected by microscopy and flow cytometry at 11 μg/ml and higher after 24 h and at 3.7 μg/ml and higher after 48 h. Thus, E7070 seemed to cause cell cycle perturbation in the G1 phase in a time- and dose-dependent manner, potentially leading to cell death. The G1 accumulation was not observed when P388 cells were treated with conventional anticancer drugs, such as 5-FU, CDDP and etoposide (data not shown).

3.2. In vitro *antitumour spectrum of E7070*

To investigate the *in vitro* antitumour spectrum of E7070, MTT colorimetric assays were performed using 42 human tumour cell lines (10 colorectal, 9 lung, 4 gastric, 4 leukaemia, 3 bladder, 3 melanoma, 3 breast, 3 prostate, 2 pancreatic and 1 renal). The activity pattern of E7070 was compared with those of seven anticancer drugs widely used in clinic. E7010, an antimitotic sulphonamide from our laboratory, was also employed. The IC_{50} values of E7070 and E7010 are illustrated in Fig. 3. E7070 showed a unique antiproliferative spectrum with a wide range of IC_{50} values. The HCT116 colorectal cancer cell line was most sensitive and NCI-H596 non-small cell lung cancer (NSCLC) cell line was most resistant to the drug, with approximately a 1000-fold difference in the IC_{50} values (0.11 and 94 μg/ml, respectively). However, most of the tumour cell lines tested were almost equally sensitive to the antimitotic sulphonamide E7010.

It has been reported that agents possessing common cellular targets or sharing similar mechanisms of action produce a similar antitumour spectrum [18]. Table 1 summarises the correlation coefficients between E7070 and other drugs, calculated by using the sets of delta values. As expected, high correlation coefficients were obtained between two topoisomerase II inhibitors, ADM and etoposide (0.77), and between the two DNA anchoring agents CDDP and MMC (0.60). In contrast, all the correlation coefficients between E7070 and each of the other drugs were lower than 0.5. Although E7070 and E7010 are both from the sulphonamide class, the correlation coefficient between them was only 0.44.

3.3. Time-dependent cytotoxic effect of E7070

The time course of E7070's cytotoxicity was monitored in human colorectal cancer HCT116 cells, using

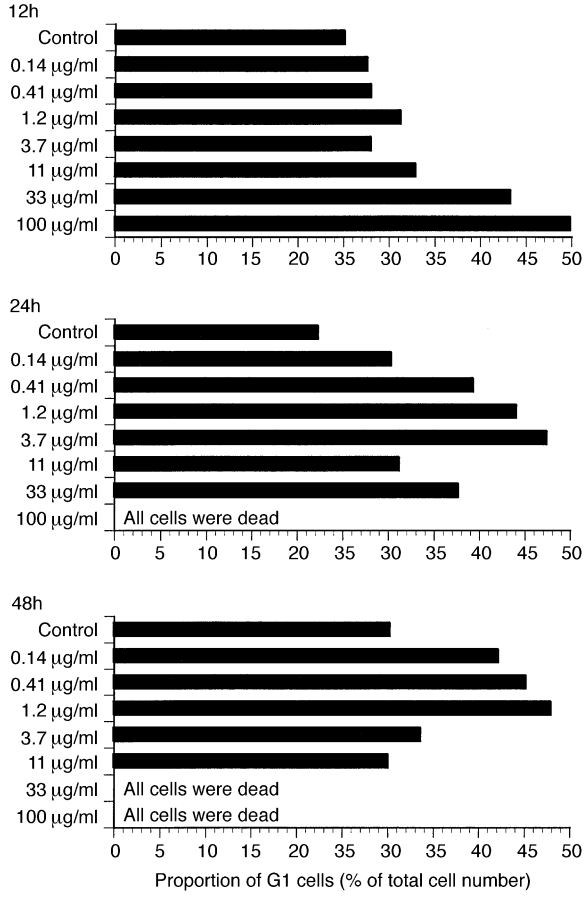

Fig. 2. The Effect of E7070 on the G1 cell proportion of P388 murine leukaemia cell line. P388 cells were incubated with indicated concentrations of E7070 for indicated hours, and proportions of the cells in the G1 phase were determined. The experiments were repeated three times and similar results were consistently obtained.

the SRB method (Fig. 4). No significant decrease in cell number was detected on day 1 at all of the doses tested. After day 1, the cytotoxic effect of E7070 was evident at doses of 1.2–33 μg/ml with a reduction in the total cell number in a time-dependent manner. Almost equal cell-killing profiles were observed within this dose range, suggesting that the efficacy of E7070 reached plateau at 1.2 μg/ml. The cell number was also decreased at 0.41 μg/ml after day 2 and at 0.14 μg/ml on day 6, while doses of 0.045 μg/ml and below exerted no effect on cell proliferation during the 6 days of treatment. The cell death induced by E7070 was found to be apoptosis because the DNA fragmentation was detected following the 2-day exposure at 1.0 μg/ml (data not shown).

Table 1
Correlation coefficients of antiproliferative activity spectrum between E7070 and eight anticancer drugs

Anticancer drug	Mechanistic class	Correlation coefficient[a] with E7070
Etoposide	Topo II inhibitor	0.48
E7010	Tubulin polymerisation inhibitor	0.44
Mitomycin C	DNA anchoring agent	0.40
Camptothecin	Topo I inhibitor	0.39
Bleomycin	DNA alkylating agent	0.37
Doxorubicin	Topo II inhibitor	0.31
5-Fluorouracil	Antimetabolite	0.28
Cisplatin	DNA anchoring agent	0.27

Topo, topoisomerase.
[a] Pearson's correlation coefficient.

3.4. In vivo *antitumour spectrum of E7070*

To examine the *in vivo* antitumour spectrum of E7070, we employed human tumour xenograft models with tumour cells showing various sensitivities to E7070 *in vitro*. Seven tumours were selected based on the rank order of IC_{50} values: (1) $IC_{50} < 0.5$ μg/ml, HCT116 (colon) and LX-1 (lung); (2) 0.5 μg/ml $\leqslant IC_{50} < 1.0$ μg/ml, SW620 (colon) and HCT15 (colon); and (3) 1.0 μg/ml $\leqslant IC_{50} < 10$ μg/ml, DLD-1 (colon), PC-9 (lung) and WiDr (colon). Antitumour efficacy was evaluated in terms of tumour regression (RTV_{min}) and tumour growth suppression (T/C) (Table 2). When administered daily for 4 days, E7070 caused not only tumour growth suppression, but also a decrease in tumour volume of the three colon cancers (HCT116, SW620 and HCT15) and two lung cancers (LX-1 and PC-9). According to the National Cancer Institute (NCI) activity criteria, these effects were judged to be active (T/C < 42%). In the HCT116 and LX-1 xenograft models, E7070 reduced tumour volume by 73–85% at the maximum tolerated dose (MTD) of 50 mg/kg/day, and tumour regression was also observed even at 25 mg/kg/day (1/2 of the MTD). In the SW620 and HCT15 xenograft models, E7070 produced clear tumour regression only at the MTD. A marginal reduction in tumour volume was obtained in PC-9 xenografts at the MTD, whereas tumour growth suppression was observed in the DLD-1 and WiDr xenografts only during the administration period.

3.5. *Dosing-schedule-dependent efficacy of E7070*

HCT116 and LX-1 xenograft models were used to investigate whether the efficacy of E7070 depends on the administration schedule. Four schedules were tested, i.e.

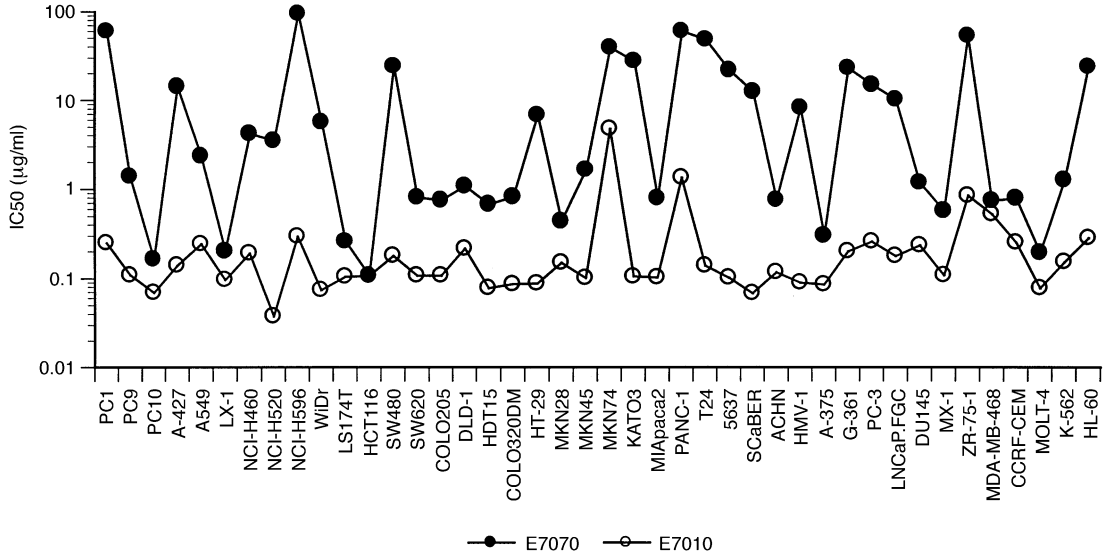

Fig. 3. The effects of E7070 and E7010 on the proliferation of 42 human tumour cell lines. Cells were treated with various concentrations of the compounds for 3 days. The antiproliferative activity was determined by the dimethylthiazolyl-2,5-diphenyltetrazolium bromide (MTT) assay, and the IC_{50} for each compound was calculated. ●, E7070; ○, E7010.

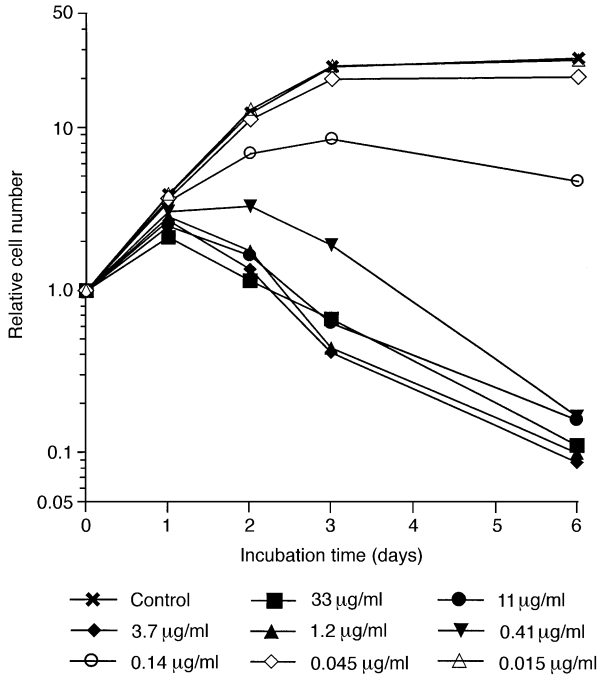

Fig. 4. Time dependency of the E7070-induced cytotoxicity. HCT116 cells were incubated at various concentrations of E7070. Relative cell number measured by the SRB method is shown on the *Y*-axis. Incubation time is shown on the *X*-axis.

single dose (QD×1), daily for 4 days (QD×4), daily for 8 days (QD×8) and every 4 days 4 times (Q4D×4). The results are summarised in Table 3. On the schedules of QD×1, QD×4 and QD×8, the total of each MTD was determined to be 200 mg/kg. Only the Q4D×4 schedule could allow the administration of a double dose (400 mg/kg). Although all four schedules caused significant tumour regression, QD×8 and Q4D×4 were obviously more effective than the QD×1 and QD×4 in both models. The former schedules, QD×8 and Q4D×4, appeared to be equally effective. In the LX-1 xenograft model, tumour-free conditions were obtained in all mice of two drug-treated groups QD×8 25 mg/kg/day and Q4D×4 100 mg/kg/day on day 22. Therefore, we continued to observe these mice for a further 5 months. Finally, eight of 10 mice and four of five mice remained tumour-free, respectively.

3.6. Side-by-side comparison of antitumour efficacy: E7070 versus 5-FU, MMC and CPT-11

The antitumour efficacy of E7070 was compared with those of three reference anticancer drugs, 5-FU, MMC and CPT-11 in the HCT116 xenograft model (Fig. 5). 5-FU and CPT-11 are two of the most widely used

Table 2
Antitumour activity of E7070 in various human tumour xenograft models on daily intravenous (i.v.) administration for 4 days (QD×4)

Xenograft model	Dose (mg/kg/day)	RTV_{min}[a]	RBW_{min}[b]	T/C value (%)	Dead[c]/treated
HCT116	12.5	0.81	–	34	0/5
colon	25	0.44	–	23	0/5
	50	0.15	0.93	8	0/5
LX-1	12.5	–	0.96	80	0/5
lung	25	0.79	–	29	0/5
	50	0.27	0.95	2911	0/5
SW620	12.5	–	–	88	0/5
colon	25	–	–	67	0/5
	50	0.63	0.91	34	0/5
	100	–	0.89	–[d]	5/5
HCT15	25	–	0.98	74	0/5
colon	50	0.46	0.88	26	0/5
PC9	12.5	–	–	89	0/5
lung	25	–	0.99	83	1/5
	50	0.93	0.95	35	0/5
	100	0.89	0.84	–[d]	5/5
DLD-1	25	–	–	83	0/5
colon	50	–	0.79	54	0/5
WiDr	12.5	–	-	85	0/5
colon	25	–	–	77	0/5
	50	–	0.95	72	0/5
	100	–	0.88	–[d]	5/5

At the termination of the experiments, the average of tumour weights±standard deviation (S.D.) of the vehicle control groups were: HCT116, 1.53±0.50; LX-1, 1.72±0.68; SW620, 1.18±0.33; HCT15, 1.21±0.37; DLD-1, 0.56±0.25; PC9, 0.47±0.15; WiDr, 0.73±0.19 g.

[a] Minimum relative tumour volume; – means no reduction of tumour volume was observed.

[b] Minimum relative body weight; – means no body weight loss was observed. The RBW_{min}s of vehicle control group in the HCT116, LX-1, HCT15 and DLD-1 xenograft models were 0.97, 0.81, 0.97 and 0.99, respectively. In control group in the SW620, PC9 and WiDr, the body weight loss was not observed.

[c] Number of dead mice during the experiment.

[d] Tumour weight was not available because all mice were dead.

Table 3
Study on the administration schedule in HCT116 human colon cancer and LX-1 human lung cancer xenograft models

Schedule	Dose (mg/kg/day)	HCT116				LX-1				
		RTV_{min}[a]	T/C[b] (%)	RBW_{min}[c]	Dead/treated	RTV_{min}[a]	T/C[d] (%)	Tumour free[e]	RBW_{min}[c]	Dead/treated
QD×1	100	0.99	32	–	0/5	N.T.[h]	N.T.[h]	N.T.[h]	N.T.[h]	N.T.[h]
	200[f]	0.37	33	0.91	0/5	0.64	46	–	0.94	0/5
QD×4	25[f]	0.52	27	0.94	0/5	0.79	30	–	–	0/5
	50[f]	0.27	13	0.90	0/5	0.27	12	–	0.95	0/5
QD×8	6.25	–	56	0.95	0/5	–	43	–	0.89	0/5
	12.5	0.22	9	0.99	0/5	0.15	5	–	–	0/5
	25[f]	0.05	1	0.95	0/5	0.03	0	8/10	0.87	1/10
	50	0.09	–[g]	0.74	5/5	N.T.[h]	N.T.[h]	N.T.[h]	N.T.[h]	N.T.[h]
Q4D×4	50	0.58	8	0.89	0/5	0.18	3	–	0.98	0/5
	100[f]	0.06	1	0.89	0/5	0.02	0	4/5	0.86	0/5
	200	0.26	–[g]	0.74	5/5	N.T.[h]	N.T.[h]	N.T.[h]	N.T.[h]	N.T.[h]

The average tumour weight ± S.D. in the control group was 1.785±0.337 g in the HCT116 xenograft model. At the termination of the experiments (day 22), the average tumour weight ± S.D. in the control group was 1777±657 mm^3 in the LX-1 xenograft model.

[a] The minimum relative tumour volume; – means that no tumour reduction was observed.

[b] Average tumour weight in treated group on day 22/average tumour weight in control group on day 22×100.

[c] The minimum relative body weight; – means that no body weight loss was observed. The RBW_{min}[s] of vehicle control group in the LX-1 xenograft model, no body weight loss was observed.

[d] Average tumour volume in treated group on day 22/average tumour volume in control group on day 22×100.

[e] Number of tumour-free mice on the end of experiment (day 153).

[f] Maximum tolerated dose on each dosing schedule. 400 mg/kg for QD×1100 mg/kg/day for QD×4, 50 mg/kg/day for QD×8 and 200 mg/kg/day for the Q4D×4 were toxic doses.

[g] Tumour weight was not available because all mice were dead.

[h] N.T., not tested.

clinical drugs against colorectal cancer [19,20]. MMC is also in common use for combination chemotherapies [21]. MMC and CPT-11 were reported to show *in vivo* antitumour effects against HCT116 [22]. Each drug was given on the reported optimal dose and administration schedule [23,24]: daily for 5 days at 19 mg/kg/day for 5-FU; single dose at 6.7 mg/kg for MMC; once every 4 days, three times, at 100 mg/kg/day for CPT-11; daily for 8 days at 12.5 (1/2 of MTD) and 25 mg/kg/day (MTD) for E7070. The MTD of E7070 demonstrated a marked decrease in tumour volume to 1% of the initial volume. It also should be noted that the T/C value was calculated as 0.6%. Furthermore, half of the MTD was still effective, reducing the tumour volume to 44% of the initial volume. MMC and CPT-11 significantly inhibited the tumour growth, as reported, with T/C values of 37.4 and 9.4%, respectively. However, no reduction in tumour volume was obtained by the administration of either MMC or CPT-11. 5-FU did not show any evidence of antitumour efficacy in this model. In conclusion, the antitumour efficacy of E7070 against HCT116 xenografts was evidently superior to those of 5-FU, MMC and CPT-11 with respect to both tumour regression and the T/C value.

4. Discussion

The antiproliferative spectrum of E7070 against a panel of 42 human tumour cell lines was different from those of seven anticancer drugs used in clinic. According to the study of the Japanese Foundation for Cancer Research, which has a database on antitumour spectrum of more than 100 anticancer drugs, there were no anticancer drugs similar to E7070 in its antitumour spectrum (data not shown). These data, together with the accumulation of cells in G1, strongly suggests that E7070 has a novel mechanism of action, because it has been reported that a comparable pattern of tumour responses can be observed among agents which have a common cellular target and share a similar mode of action [18]. We understand that the investigation into the mechanism of action is one of the most important issues. Therefore, in order to find molecular targets and drug-sensitive cellular pathways, we are currently carrying out DNA microarray analysis and trying to identify binding proteins. Thus far, we have discovered that E7070 downregulates both mRNA and protein levels of cyclin H [25], which is an essential component of CAK. We believe that the effect on cyclin H expression may be involved in the inhibition of the G1/S transition (data not shown).

The unique antitumour spectrum of E7070 may warrant the potential utility of this drug in the clinic for the treatment of tumours that are refractory to current anticancer chemotherapies. In this aspect, it should be noted that E7070 caused a significant decrease in tumour volume in the human colon cancer HCT116 xenograft model, whereas MMC and CPT-11 showed only tumour growth suppression without any tumour

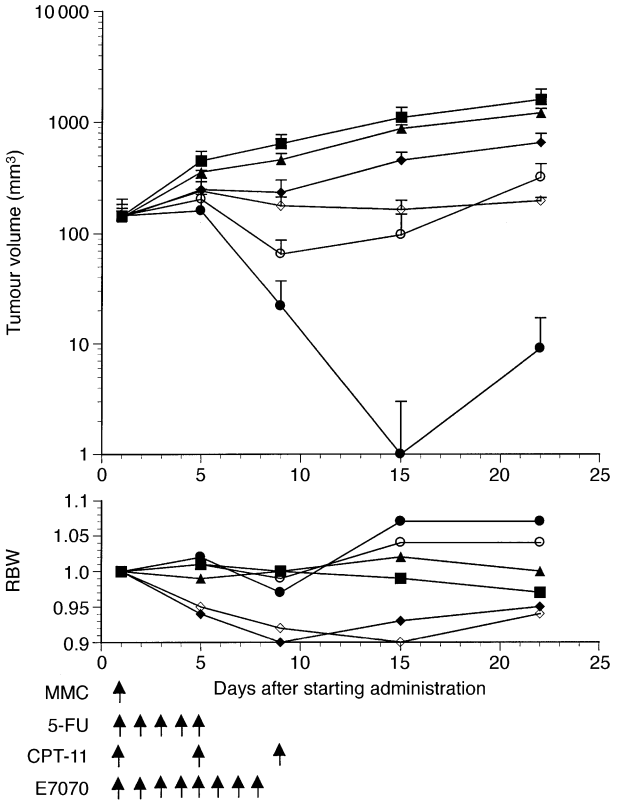

Fig. 5. Comparison of the antitumour efficacy of E7070 with those of other anticancer drugs in the HCT116 xenograft model. Growth curves of tumour and changes in relative body weight (RBW) in the untreated group (■), groups treated with E7070 (25 and 12.5 mg/kg/day for QD×8; ● and ○), 5-FU (19 mg/kg/day for QD×5; ▲), MMC (6.7 mg/kg for QD×1; ◆) and CPT-11 (100 mg/kg/day for Q4D×3; ◇) are shown. Error bars indicate the standard deviations (S.D.).

regression. Furthermore, E7070 was active against human colon cancer HCT15 xenografts which have been found to be considerably resistant to various drugs such as paclitaxel, CDDP, 5-FU and ADM [26]. These data also indicate another possibility that the efficacy of E7070 is independent of the multi-drug resistance (MDR) phenotype, because HCT15 and HCT116 cells are known to highly express Pgp and LRP/MRP, respectively [27,28].

In experiments using HCT116 cells, E7070 was found to decrease the expression of cyclin E and the phosphorylation of cdk2, both of which are essential for G1/S transition [29]. The accumulation of p53 in the same cell line was also evident after 48 h of drug exposure (data not shown). To explain these observations, serious efforts to identify the molecular target of E7070 are in progress. Our current research efforts are also directed towards what kinds of genetic backgrounds in cancer are linked with a sensitivity to E7070. Because HCT116, one of the most sensitive cell lines *in vitro* and *in vivo*, possesses mutated *ras* and wild-type (wt) *TP53*, the relationship between drug sensitivity and the status of these two genes was surveyed using published data [30,31]. As for *TP53*, some of the highly sensitive cell lines (HCT116, LS174T and MOLT-4) have wt *TP53* and there are no sensitive cell lines with null or truncated mutations of *TP53*. However, H460 and A549 with wt *TPp53* were more than 10-fold less sensitive to E7070 compared with the highly sensitive cell lines such as HCT116 and LS174T. Furthermore, cell lines with point mutations in *TP53* showed a wide range of sensitivities. MOLT-4 with mutated *ras* was fairly sensitive to E7070, as were HCT116, A427 and SW480 which also possess mutated ras. Therefore, the status of both genes appears to be unrelated to drug sensitivity. For further studies, gene expression analysis with microarrays and quantitative real-time polymerase chain reaction (PCR) will be conducted.

The *in vitro* and *in vivo* studies showed that the antitumour efficacy of E7070 on QDx4 in seven human tumour xenograft models were well correlated with the antiproliferative activity. A high correlation ($r^2 = 0.86$) was obtained between T/C values *in vivo* and IC_{50} values in the MTT assay. Although the number of tumours tested were rather limited, these data may support the idea that the *in vitro* susceptibility test in the clinic would afford a good prediction of the clinical response of E7070. With respect to the relationship between the *in vitro* and *in vivo* data, there is another point to be discussed. In the *in vitro* SRB assay using HCT116 cells, the cytotoxic effect of E7070 emerged from 0.14 μg/ml and reached a plateau at concentrations of 1.2 μg/ml and over. Extending the drug exposure time resulted in an increase of the cell-killing effect. In light of these observations, the optimal *in vivo* antitumour efficacy should be achieved by maintaining plasma concentrations in an adequate range corresponding to 0.14–1.2 μg/ml *in vitro* as long as possible. This consideration may explain why the QD×8 schedule was superior to the QD×1 and QD×4 schedules despite the total dose intensity being equally 200 mg/kg.

Because of its unique and promising antitumour profiles, E7070 has progressed to phase I clinical trials in collaboration with the European Organization for Research and Treatment of Cancer (EORTC). Furthermore, after determination of the dose-limiting toxicities and safety doses, phase II trials have just started in Europe.

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