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# Antitumor Activity of a Novel Quinoline Derivative, TAS-103, with Inhibitory Effects on Topoisomerases I and II

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**A novel quinoline derivative, TAS-103 (6-[[2-(dimethylamino)ethyl]amino]-3-hydroxy-7*H*-indeno[2,1-*c*]quinolin-7-one dihydrochloride), was developed as an anticancer agent targeting topoisomerases (topo) I and II, with marked efficacy in solid tumors. TAS-103 inhibited topo I and II ($IC_{50}$: 2 $\mu M$, 6.5 $\mu M$) at a concentration similar to or lower than those of previous agents, and had a strong cytotoxic effect on P388 and KB cells ($IC_{50}$: 0.0011 $\mu M$, 0.0096 $\mu M$). TAS-103 stabilized topo I and II-DNA cleavable complexes in KB cells, generating a similar amount of topo II-DNA complex to that induced by etoposide (VP-16) but a smaller amount of topo I-DNA complex than that produced by camptothecin (CPT). In the *in vivo* study, intermittent i.v. administration was markedly effective against s.c.-implanted murine tumors. Furthermore, TAS-103 had marked efficacy against various lung metastatic tumors, and a broad antitumor spectrum in human tumor xenografts (derived from lung, colon, stomach, breast, and pancreatic cancer). The efficacy of TAS-103 was generally greater than that of irinotecan (CPT-11), VP-16, or *cis*-diamminedichloroplatinum (CDDP).**

Key words: TAS-103 — Quinoline derivative — Antitumor activity — Topoisomerase I — Topoisomerase II

Topoisomerases (topo) are enzymes that relieve the secondary twist on the DNA strand in the process of DNA synthesis and transcription via breakage, passage and reunion of the DNA strand.[1,2] The enzymes are important targets for various chemotherapeutic agents. The sensitivity of tumor cells to topo inhibitors is strongly associated with intranuclear topo levels.[3–5] The expression patterns of topo I and II differ. Topo I expression shows no major changes during the cell cycle, whereas topo II expression increases in response to DNA replication, reaches a maximum at the G2-M phase, and then rapidly decreases.[6,7] Furthermore, the expression levels of the two enzymes vary with the cancer type. The expression of topo I is marked in colorectal cancer, prostate cancer, ovarian cancer and malignant lymphoma, but low in kidney cancer.[8,9] On the other hand, topo II is markedly expressed in breast cancer, ovarian cancer and malignant lymphoma.[10–12] Thus, we speculated that an agent inhibiting both topo I and II would have a broader anticancer spectrum than an agent inhibiting either one alone.

The acquisition of multidrug resistance (MDR) in cancer is a major obstacle in the management of cancer, and overcoming clinical MDR is a prime concern in cancer chemotherapy. At least two types of MDR can be distinguished on the basis of the mechanisms involved. Classical MDR is caused by reduced intracellular drug levels due to the expression of the membrane bound P-glycoprotein efflux pump. Atypical MDR is caused by reduced topo II expression,[13,14] and there is cross resistance to a variety of topo II inhibitors. However, atypical MDR tumors show no cross resistance to topo I inhibitors. Furthermore, in some of these tumors, the expression level of topo I is reportedly increased and the sensitivity to topo I inhibitors is enhanced.[15] A similar phenomenon has been observed in tumors that became resistant to topo I inhibitors, suggesting that topo I and II can compensate for each other's function.[16] These findings suggest that inhibition of topo I and II using a single agent would overcome the resistance to monospecific topo inhibitors and prevent the appearance of MDR.

Recently, several compounds (RP-60475, Saintopin, Fagaronine, AzaIQD) have been found to inhibit topo I and II.[17–20] Among them, RP-60475 (Intoplicine) is under clinical trial. We examined the topo I, topo II, and topo I and II inhibitors previously introduced, and synthesized various compounds related to their common structure. We selected compounds satisfying the following criteria: (1) inhibition of both topo I and II, (2) a strong cytotoxic effect, (3) high selectivity for solid tumors *in vivo*. TAS-103 (Fig. 1) satisfies these conditions and in addition has satisfactory metabolic and toxicologic characteristics. Herein, we describe the topo I- and II-inhibiting capacity, cytotoxic effect *in vitro*, efficacy in various mouse tumors *in vivo*, and efficacy in human tumors, as well as various lung metastatic tumors, of TAS-103.

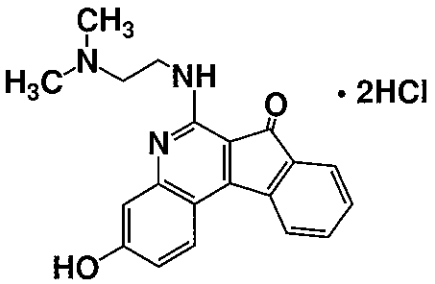

Fig. 1. Structure of TAS-103, 6-[[2-(dimethylamino)ethyl]-amino]-3-hydroxy-7*H*-indeno[2,1-*c*]quinolin-7-one dihydrochloride.

## MATERIALS AND METHODS

**Agents** TAS-103, CPT-11, SN-38 and RP-60475 were synthesized by Taiho Pharmaceutical Co., Ltd. VP-16 and CDDP were purchased from Nippon Kayaku Co., Ltd. For *in vivo* experiments, VP-16 was dissolved in saline containing 6.5% Tween 80 and 3.5% dimethyl sulfoxide. The other agents were dissolved in 5% glucose solution or saline.

**Animals** Specific pathogen-free male BDF1, C57BL/6, BALB/c, B6C3F1, C3H/HeN, and BALB/c *nu/nu* mice were obtained commercially. Animals were maintained under pathogen-free conditions and were age-matched (6–10 weeks of age) at the onset of each experiment.

**Tumor cells** Murine tumor cell lines, i.e., P388 leukemia, B16-BL6 melanoma, UV2237M fibrosarcoma, and K1735M2 melanoma cells were passaged and maintained *in vitro* in RPMI1640 containing 10% fetal calf serum in a humidified atmosphere of 5% $CO_2$ in air. UV2237M and K1735M2 cells were kindly supplied by Dr. Fidler, U.T. M.D. Anderson Cancer Center, Houston, TX. For *in vivo* experiments, Lewis lung carcinoma (LLC), B16-BL6, and Colon 26 cells were maintained in an inbred mouse strain of tumor origin. Human non-small cell lung cancers (NSCLC) (Lu-99, LC-6, and LX-1), a gastric cancer (MKN-45), a colon cancer (COL-5), a mammary cancer (MX-1) and a pancreatic cancer (PAN-6) were maintained s.c. in BALB/c *nu/nu* mice.

**Topo I and II catalytic activity assay** The topo I catalytic activity was measured by the relaxation assay using pBR322 DNA as described previously.[21] Supercoiled pBR322 DNA (0.25 $\mu$g), agent and 1 unit of topo I (Takara Shuzo Co., Ltd.) were added to Tris-HCl buffer (35 m*M* Tris-HCl, pH 8.0, 72 m*M* KCl, 5 m*M* $MgCl_2$, 5 m*M* dithiothreitol, 5 m*M* spermidine, 0.01% bovine serum albumin, BSA) to a total volume of 20 $\mu$l. After incubation at 37°C for 30 min, 5 $\mu$l of reactive inhibition solution (2.5% sodium dodecyl sulfate (SDS), 0.05% bromophenol blue, 50% glycerol) was added to stop the reaction. The reactive solution was applied to 1% agarose gel, and electrophoresis was performed in TBE buffer (90 m*M* Tris-borate, pH 8.3, 2.5 m*M* EDTA) (Mupid 2 gel system). After the gel was removed, it was stained in 2 $\mu$*M* ethidium bromide (EtBr) for 30 min, and then photographed under ultraviolet light using Polaroid film. The inhibitory effect of the agent was expressed as the dose ($IC_{50}$) inhibiting the conversion of supercoiled DNA into the relaxed form by 50% compared to the control group.

Crude nuclear extracts containing topo II were prepared from KB cells, and topo II catalytic activity was measured by the decatenation assay using kinetoplast DNA as described previously.[22] Kinetoplast DNA (0.25 $\mu$g) (TopoGen Inc.), a test agent and topo II were added to the standard reaction mixture [50 m*M* Tris-HCl, pH 7.5, 8.5 m*M* KCl, 10 m*M* $MgCl_2$, 0.5 m*M* dithiothreitol, 0.5 m*M* EDTA), ATP (1 m*M*) and BSA (30 $\mu$g/ml)] to a total volume of 20 $\mu$l. After incubation at 37°C for 30 min, the reactions were terminated with 5 $\mu$l of the reactive inhibition solution. Samples were then electrophoresed in 1% agarose with TBE buffer (Mupid 2 gel system). Gels were stained with 2 $\mu$*M* EtBr for 30 min. DNA bands were visualized by UV transillumination and photographed using Polaroid film. Inhibitory activity was calculated from the analysis of the film. The $IC_{50}$ value was defined as the drug concentration needed to produce a 50% reduction in the amount of minicircle DNA relative to the control.

**Detection of topo I- or II-DNA cleavable complex** The procedure was performed as described[23] with some modifications. KB cells ($2.0–2.5 \times 10^6$ cells) were treated with drugs in serum-free medium for 1 h at 37°C. After removal of the medium, the cells were lysed with 1 ml of 1% Sarkosyl in TE buffer. The lysate was layered on a stepwise CsCl gradient (density: 1.82, 1.72, 1.50, 1.37 g/ml from the bottom) in polyallomer tubes (13×51 mm, Beckman). The gradient was centrifuged in an SW50.1 rotor at 31,000 rpm for 16 h at 25°C and fractionated into 0.2 ml fractions. The fractions were diluted with 0.4 ml of 25 m*M* sodium phosphate buffer (pH 7.0) and their DNA concentration was measured by means of a rapid fluorescence-based assay using Hoechst 33342.[24] Free or bound topo I and II in the fractions were detected by immunoblotting with anti-human topo I and II IgG (1 : 2500 dilution, TopoGen Inc.), respectively, on a nitrocellulose filter (0.45 $\mu$m, 8×12 cm, Pierce) using a dot blot device (Pierce). After incubation with $^{125}$I-labeled protein A, the enzyme dot was analyzed by a BAS-2000 system (Fuji Film).

***In vitro* antitumor activity evaluation** Tumor cells were plated at the density of $1 \times 10^3$/38 mm$^2$ well in flat-bottomed microtest III plates (Falcon Plastics, Oxnard,

CA) and cultured overnight. The cells were incubated with the test compound at various concentrations for 72 h. After completion of culture, the cells were fixed with glutaraldehyde for 15 min and washed 3 times with water. They were stained with 100 $\mu$l of 0.05% crystal violet solution for 15 min and washed 3 times with water. Crystal violet was eluted with 0.05 *M* $NaH_2PO_4$/ethanol (1 : 1 v/v) and the optical density was measured by an automated spectrophotometric plate reader at a single wavelength of 540 nm. The $IC_{50}$ value was defined as the drug concentration needed to produce a 50% reduction of optical density relative to the control.

***In vivo* antitumor activity against s.c.-implanted mouse Colon 26 adenocarcinoma** Fragments ($2\times2\times2$ mm$^3$) of Colon 26 adenocarcinoma were implanted s.c. into BALB/c mice (day 0). TAS-103 was intravenously administered with a single (qdx1), consecutive (qdx5), or intermittent (q4dx3) schedule at various doses from Day 1. The mice were weighed twice a week to monitor toxic effects. The body weight reduction ($\Delta$BW) due to drug treatment was calculated according to the following formula:

$$\Delta \text{BW (g)} = (\text{mean BW on day } n) - (\text{mean BW on the day administration was started})$$

The mice were killed on day 15, and the tumors were removed and weighed. The antitumor activity was evaluated as the percentage of tumor weight inhibition (TWI) compared to the mean tumor weight in the control group, according to the following formula.

$$\text{TWI } \% = (1 - T/C) \times 100$$

$T$ and $C$ represent the mean tumor weight in the test group and the control group, respectively.

***In vivo* antitumor activity against LLC growing s.c or in lung in mice** BDF1 mice were implanted s.c. with $2\times2\times2$ mm$^3$ tumor fragments or injected through the tail vein with $5\times10^5$ LLC cells to induce s.c. or lung tumor, respectively (day 0). TAS-103 was administered i.v. with a q4dx3 schedule from day 3. The control drugs CPT-11 and VP-16 were administered i.v. at the approximate maximum tolerated dose (MTD) with a q4dx3 and qdx5 schedules, respectively. CDDP[25,26] was administered i.v. at the clinically equivalent dose (CED) in normal mice with a qdx1 schedule. The mice were weighed twice a week to monitor toxic effects. The mice with s.c. tumor were killed on day 14, and the tumors were removed and weighed. The antitumor effect of the agent was evaluated in terms of TWI (%), as described above. In mice with lung tumor, the survival period (days) was monitored and the antitumor activity was evaluated as the percentage of increase in life span (ILS) compared to the mean survival days of mice in the control group according to the following formula.

$$\text{ILS } \% = (T/C - 1) \times 100$$

$T$ and $C$ represent the mean survival days in the test and control group, respectively. Mice that survived for more than 60 days were considered cured.

**Antitumor activity against mouse experimental lung metastatic tumors (K1735M2)** K1735M2 melanoma cells ($2\times10^5$) maintained *in vitro* were implanted into B6C3F1 mice through the tail vein (day 0). TAS-103 was administered i.v. with a single (qdx1), consecutive (qdx5), or intermittent (q4dx3, q7dx3, q10dx2) schedule at various doses from day 1. The survival period (day) of the mice was monitored, and the antitumor activity was evaluated as ILS (%).

**Antitumor activity against mouse experimental lung metastatic tumors (B16-BL6, UV2237M)** B16-BL6 melanoma and UV2237M fibrosarcoma cells ($1\times10^5$) maintained *in vitro* were implanted into C57/BL6 and C3H/HeN mice through the tail vein, respectively (day 0). The test agents were administered i.v. according to the optimal schedule from day 1 (B16-BL6) or day 7 (UV2237M). Four weeks after inoculation, the lungs were excised from the mice and fixed in 10% formalin. The number of pulmonary metastases was counted under a dissecting microscope. The antitumor activity was calculated as the percentage inhibition rate (IR) relative to the mean number of pulmonary metastases in the control group.

$$\text{IR } \% = (1 - T/C) \times 100$$

$T$ and $C$ represent the mean number of pulmonary metastases in the test and control group, respectively.

***In vivo* antitumor activity against human tumor** A fragment ($2\times2\times2$ mm$^3$) of a human tumor (NSCLCs Lu-99, LX-1 and LC-6, colon cancer COL-5, stomach cancer MKN-45, breast cancer MX-1, or pancreatic cancer PAN-6) was implanted s.c. into BALB/c nude mice. When the tumor mass had reached between 50 and 300 mm$^3$, the mice were divided into experimental groups consisting of at least 7 mice/group (day 0). The test agents were administered i.v. according to the optimal schedule at the approximate MTD (TAS-103, CPT-11, and VP-16) or the CED (CDDP)[25,26] from day 0. The tumor volume and body weight were measured twice a week to monitor the antitumor effect and the toxicity potential of the test agent. Twenty-eight days after the start of administration, the mice were killed, and the tumors were removed and weighed. The antitumor activity was calculated as TWI (%).

**Statistical evaluation** Welch's $t$ test or Wilcoxon's exact test was used for statistical evaluation. The global first kind of error in these tests was controlled by using a closed testing procedure[27] in comparison with the control group or Hochberg's sequentially rejective Bonferroni procedure[28] in other comparisons.

RESULTS

**Inhibition of topo I and II by TAS-103** The inhibitory effects of TAS-103 on topo I and II were compared with those of the control drugs (Table I). TAS-103 inhibited the relaxation of supercoiled DNA caused by topo I at 2 $\mu M$, and manifested an activity similar to that of CPT, SN-38 (the active metabolite of CPT-11), or RP-60475. In addition, TAS-103 inhibited the decatenation of kinetoplast DNA caused by topo II at 6.5 $\mu M$, and had an approximately 12-fold higher activity than VP-16 (80 $\mu M$). RP-60475 inhibited topo II at a slightly lower concentration (2 $\mu M$) than TAS-103.

Since classical topo I and II inhibitors such as CPT and VP-16, respectively, induce antitumor activity through the stabilization of topo-DNA complexes (cleavable complexes), we next studied whether TAS-103 induces cleavable complexes in cells. In the K/SDS precipitation assay,[29] TAS-103 induced topo-DNA complexes in a dose-dependent manner, as did CPT and VP-16 (data not shown). Therefore, topo-DNA complexes were further separated with CsCl stepwise gradients, and examined by immunoblotting using a specific anti-topo I or topo II antibody. As shown in Fig. 2A, TAS-103 (3 $\mu M$) apparently stabilized topo I-DNA cleavable complexes in KB cells, as detected in terms of DNA peaks (fractions 18–22), although to a lesser extent than CPT (3 or 30 $\mu M$). On the other hand, TAS-103 (3 $\mu M$) induced an amount of topo II-DNA cleavable complexes similar to that induced by VP-16 (30 $\mu M$) (Fig. 2B). RP-60475 induced both topo I- and topo II-DNA cleavable complexes to only a slight extent (data not shown). These findings indicated that TAS-103 stabilized both topo I- and topo II-DNA cleavable complexes, although to different extents.

***In vitro* cytotoxic activity of TAS-103** The $IC_{50}$ values, a measure of the *in vitro* cytotoxicity, of TAS-103 for P388 and KB cells were 0.0011 and 0.0096 $\mu M$, respectively, being similar to those of SN-38 (0.0059, 0.0046 $\mu M$) (Table II). TAS-103 was cytotoxic to tumor cells at lower concentrations than CPT-11 (0.18, 0.11 $\mu M$), VP-16 (0.029, 0.39 $\mu M$) and RP-60475 (0.017, 0.31 $\mu M$).

Table I. Inhibitory Activity of TAS-103 against Topoisomerases I and II

| Compound | $IC_{50}$ ($\mu M$) | |
|---|---|---|
| | Topo I[a] | Topo II[b] |
| TAS-103 | 2 | 6.5 |
| CPT-11 | 80 | — |
| SN-38 | 2 | — |
| CPT | 2 | — |
| VP-16 | — | 80 |
| RP60475 | 2 | 2 |

*a*) Relaxation assay using pBR322 DNA.
*b*) Decatenation assay using Kinetoplast DNA.
—: Not tested.

**Antitumor activity of TAS-103 against s.c.-implanted mouse Colon 26 adenocarcinoma** We next determined the *in vivo* antitumor activity of TAS-103 against s.c.-implanted mouse tumor. TAS-103 markedly inhibited tumor growth at the maximal dose in subcutaneously implanted Colon 26 tumors, with 85% inhibition after a single administration (qdx1), 89% after administration for 5 consecutive days (qdx5), and 97% after three intermittent administrations at 4-day intervals (q4dx3) (Fig. 3). Comparison among different schedules revealed that the greatest inhibitory effect occurred after intermittent administration; even in the low-dose range, the effect was greater than in other schedules. Furthermore, body weight loss (an index of toxicity) was most marked after continuous administration. Thus, intermittent administration was the most effective of the three schedules, followed by single and then by continuous administration.

To compare the antitumor activity of TAS-103 with that of the control drugs, CPT-11 and VP-16 at the approximate MTD, or CDDP at the CED, were administered with the optimal schedule.[25,26,30,31] CPT-11, VP-16, and CDDP showed inhibition rates of 56%, 66%, and 50%, respectively.

**Antitumor activity of TAS-103 against LLC growing sc or in lung in mice** Recent reports indicated that the organ environment has profound effects on the response of tumor cells to chemotherapy.[32,33] Thus, s.c.-implanted tumor models have limitations for studies of the interaction of tumor cells with organ environments and the organ distribution of drugs. Preliminary disposition studies using various animals suggested that TAS-103 is preferentially distributed in lung, stomach, large intestine, etc. (data not shown). Therefore, the antitumor activity of TAS-103 was determined against LLC growing in lung as well as the tumor growing s.c. in mice. TAS-103 strongly inhibited tumor growth at the optimum dose in s.c.-implanted LLC, by 99% (Table III). Three of the 7 mice were cured. The control drugs also inhibited tumor growth. The inhibition rates of CPT-11, VP-16 and CDDP were 93% (2 of the 7 mice were cured), 78% and 51%, respectively. In addition, all the mice with lung tumor of LLC were cured after TAS-103 administration at the optimum dose. However, the ILS values of CPT-11, VP-16 and CDDP were 38%, 62% and 24%, respectively. TAS-103 showed a greater life-prolonging effect than the control drugs. Thus, TAS-103 was not only effective in s.c.-transplanted tumors, but also strongly active against lung cancer orthotopically reconstructed in the lung.

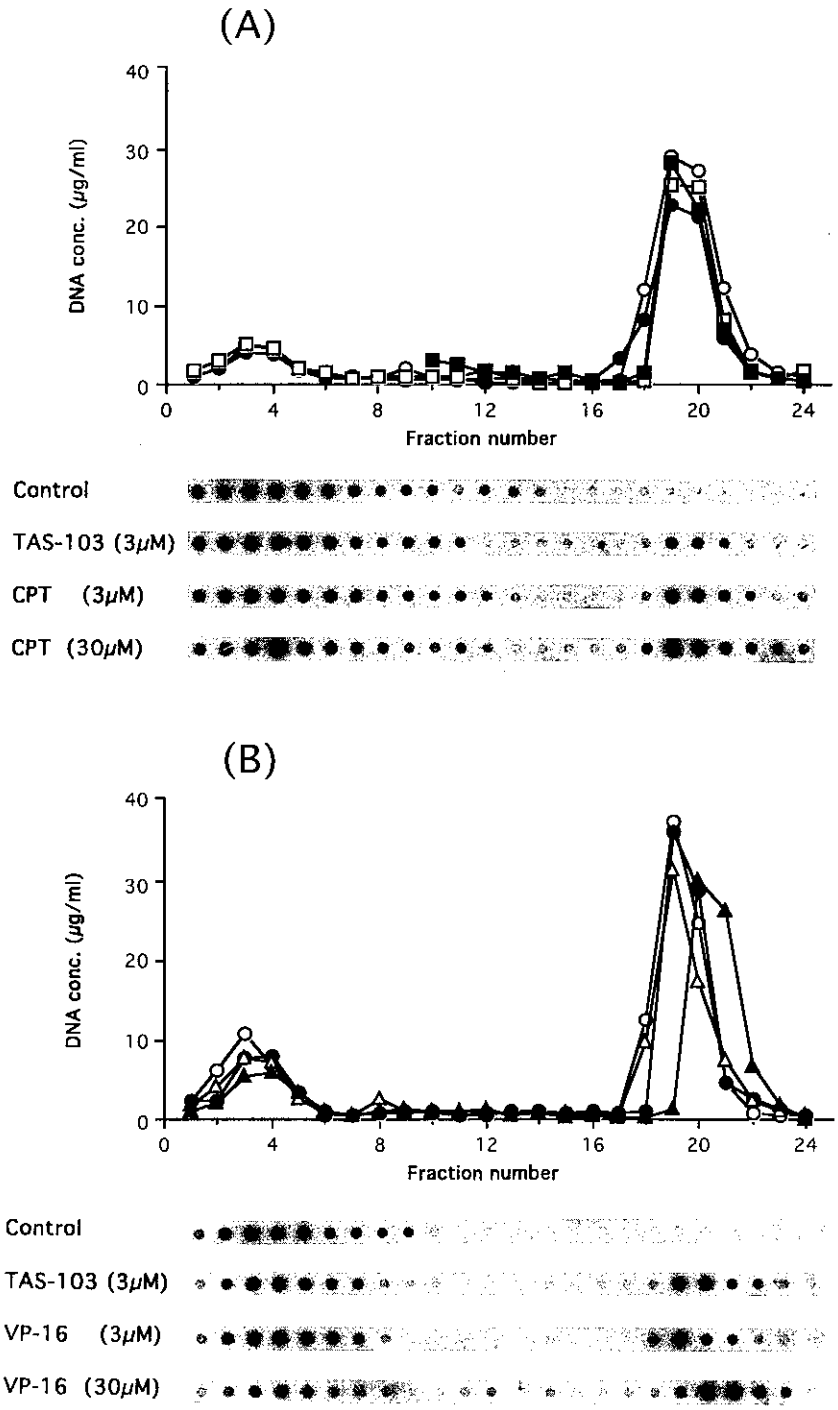


Fig. 2. Topo I- and topo II-cleavable complex formation by TAS-103 in KB cells. KB cells were treated with medium alone (○), TAS-103 (3 $\mu M$, ●), CPT (3 $\mu M$, □; 30 $\mu M$, ■), or VP-16 (3 $\mu M$, △; 30 $\mu M$, ▲) for 1 h. The cells were lysed with 1% Sarkosyl in TE buffer. The lysate was layered on a stepwise CsCl gradient and ultracentrifuged for 16 h. The gradient was fractionated into 24 fractions. The DNA concentration ($\mu$g/ml) in the fractions was measured by rapid fluorescence-based assay using Hoechst 33342 (34). Free (fractions 1–10) and bound (fractions 18–22) topo I (A) or topo II (B) in the fractions were detected by immunoblotting with anti-human topo I or II IgG (1 : 2500 dilution) on a nitrocellulose filter. The enzyme dot was detected with $^{125}$I-labeled protein A and analyzed with a BAS-2000 system. The DNA concentration in fractions 1–10 for 30 $\mu M$ CPT was not determined due to nonspecific fluorescence of CPT.

**Antitumor activity of TAS-103 against lung metastasis tumors** We further examined the antitumor activity of TAS-103 against lung metastatic tumors of K1735 M2 melanoma with various administration schedules (Fig. 4). TAS-103 administered for 5 consecutive days (qdx5) prolonged the life span, with an ILS of 79% (maximum), while a single administration of TAS-103 (qdx1) resulted in an ILS of 78%. Intermittent administration at intervals of 4 (q4dx3), 7 (q7dx3) and 10 (q10dx2) days prolonged the life span more effectively, with ILS values of 109%, 129% and 128%, respectively. The life-prolonging effect of TAS-103 was enhanced after intermittent administration relative to consecutive or single administration, as in s.c.-implanted Colon 26 tumors. The values of the ratio of the dose showing ILSmax to that showing ILS50%, that is, ILSmax/ILS50%, were 1.87 (qdx5), 1.97 (qdx1), 2.27 (q4dx3), 3.08 (q7dx3) and 2.70 (q10dx2). The range of efficacy was widest after intermittent administration. Of the intermittent administration schedules, q7dx3 appeared the most appropriate with a wider range of efficacy, a slightly higher maximal efficacy, and a relatively smaller body weight loss at a maximal dose (q4dx3 +0.35 g, q7dx3 −1.5 g, q10dx2 −3.2 g), than q4dx3 and q10dx2. The ILS values of CPT-11, VP-16, and CDDP were 23%, 39%, and 59%, respectively. The efficacy of TAS-103 was significantly higher than those of the control drugs.

Table II. *In vitro* Cytotoxic Activity of TAS-103 against P388 and KB Cells

| Compound | $IC_{50}$ ($\mu M$) | |
|---|---|---|
| | P388 | KB |
| TAS-103 | 0.0011 | 0.0096 |
| CPT-11 | 0.18 | 0.11 |
| SN-38 | 0.0059 | 0.0046 |
| VP-16 | 0.029 | 0.39 |
| RP60475 | 0.017 | 0.31 |

P388 and KB cells ($1\times10^3$) seeded in a 96-well microplate were incubated with the compounds for 3 days. The $IC_{50}$ value was determined by crystal violet dye exclusion assay.

In addition, TAS-103 inhibited the number of pulmonary metastatic foci of B16-BL6 melanoma by 82%; CDDP inhibited it by 70% and CPT-11 had no efficacy (Table IV). Furthermore, TAS-103 showed a strong inhibitory effect on advanced pulmonary metastases of UV2237M fibrosarcoma (99%), being significantly more effective than CPT-11 (54%), VP-16 (69%) and CDDP (66%).

**Antitumor activity of TAS-103 against human tumor xenografts** Human tumor xenografts are one of the best models for predicting drug efficacy in clinical settings.[34] The effects of TAS-103 on human tumor xenografts were examined using NSCLCs (Lu-99, LX-1, LC-6), a colon cancer (COL-5), a stomach cancer (MKN-45), a breast cancer (MX-1) and a pancreatic cancer (PAN-6) (Table V). TAS-103 inhibited tumor growth by 52% to 84% for

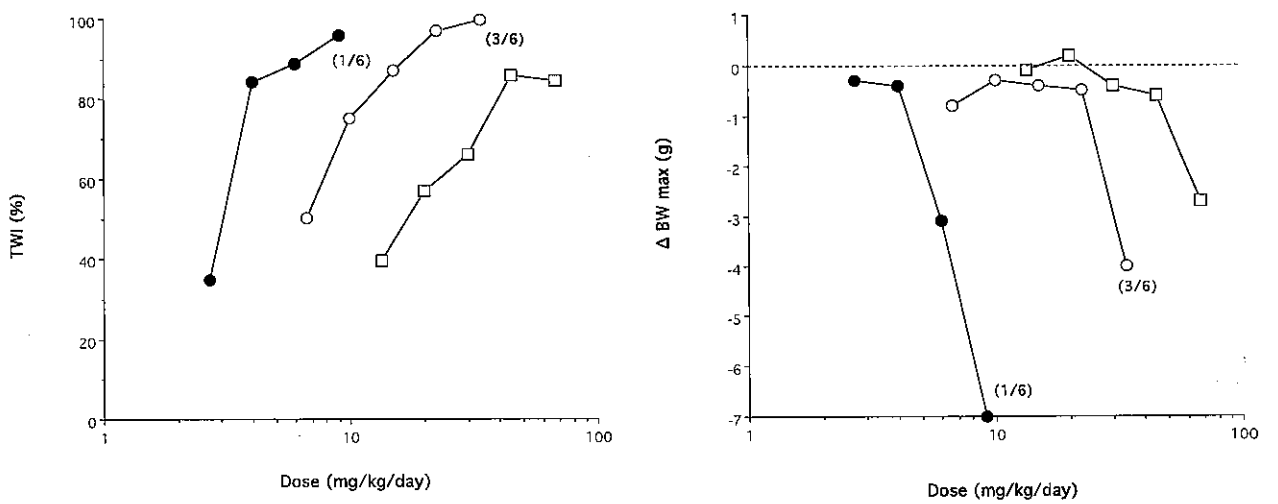


Fig. 3. Antitumor activity of TAS-103 against s.c.-implanted Colon 26 in BALB/c mice. BALB/c mice were implanted s.c. with a $2\times2\times2$ mm$^3$ fragment of Colon 26 adenocarcinoma on day 0. TAS-103 was administered i.v. from day 1 on schedules of q4dx3 (○), qdx5 (●) and qdx1 (□). The mice were killed on day 15, and the tumors were removed and weighed. The TWI% compared to the mean tumor weight in the untreated control group and maximal rate of body weight reduction (Δ BWmax, g) from day 5 to day 12 were evaluated. The number of animals that died due to toxicity of TAS-103 per group is given in parenthesis.

Table III. Antitumor Activity of TAS-103 against LLC Growing s.c. or in Lung in Mice[a)]

| Location of tumor | Treatment | Schedule | Dose (mg/kg/day) | TWI (%) | ILS (%) | Cured mice | Δ $BW_{max}$ (g)[b)] Test/Control |
|---|---|---|---|---|---|---|---|
| s.c. | Control | — | 0 | — | — | 0/7 | |
| | TAS-103 | q4dx3 | 7.5 | 79** | — | 0/7 | +0.4/+0.1 |
| | | | 13 | 94** | — | 3/7 | +0.1/+2.5 |
| | | | 22.5 | 99** | — | 3/7 | −1.3/+2.5 |
| | CPT-11 | q4dx3 | 50 | 93** | — | 2/7 | −0.1/+0.1 |
| | VP-16 | qdx5 | 10 | 78** | — | 0/7 | −0.5/+2.0 |
| | CDDP | qdx1 | 7 | 51* | — | 0/7 | −1.4/+0.1 |
| Lung | Control | — | 0 | — | — | 0/7 | |
| | TAS-103 | q4dx3 | 7.5 | — | 91*** | 0/7 | −0.7/−0.3 |
| | | | 13 | — | >155*** | 5/7 | −1.1/−0.3 |
| | | | 22.5 | — | >161*** | 7/7 | −2.7/+2.0 |
| | CPT-11 | q4dx3 | 50 | — | 38*** | 0/7 | −1.1/−0.3 |
| | VP-16 | qdx5 | 10 | — | 62*** | 0/7 | −0.5/−0.3 |
| | CDDP | qdx1 | 7 | — | 24* | 0/7 | −1.4/−0.3 |

*a*) BDF1 mice were implanted s.c. with a 2×2×2 $mm^3$ fragment or through the tail vein ($5\times10^5$ cells) with LLC on day 0. The test agents were administered i.v. from day 3. The mice with s.c. tumor were killed on day 14, and the tumors were removed and weighed. The antitumor activity was evaluated as % TWI relative to the mean tumor weight of the untreated control group. For the mice with lung tumor, the antitumor activity was evaluated as ILS%. Mice that had no tumor or survived for more than 60 days were considered cured.

*b*) Maximal rate of body weight reduction (Δ $BW_{max}$, g) in the drug-treated group is shown in comparison with Δ BW in untreated control mice on the same day.

* $P<0.05$, ** $P<0.01$, *** $P<0.001$ by two-tailed Welch's $t$ test for TWI and Wilcoxon's exact test for ILS as compared to the control group.

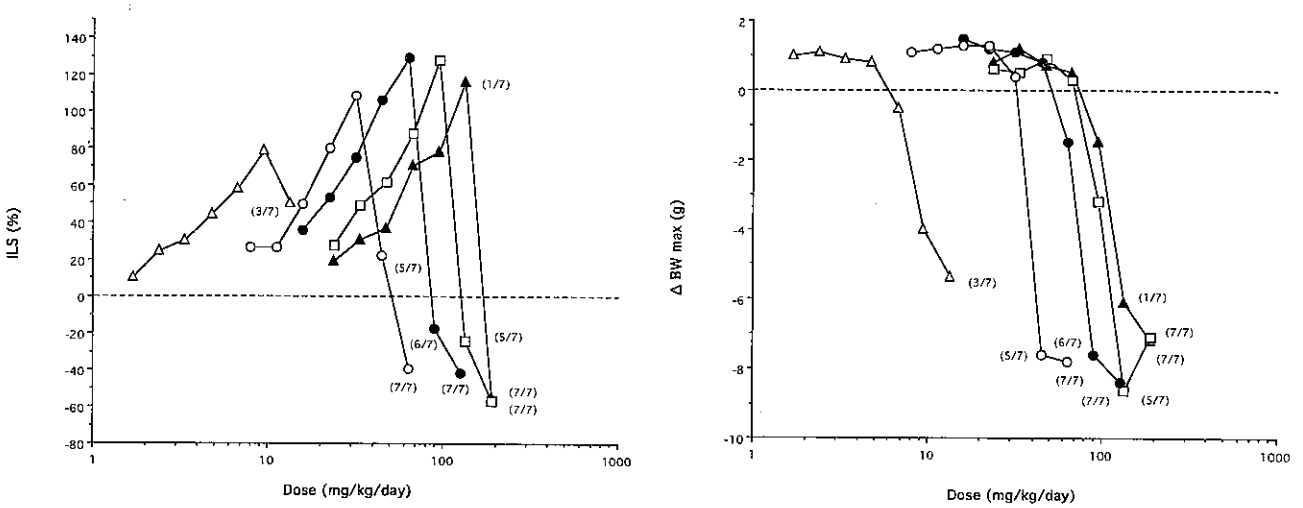


Fig. 4. Antitumor activity of TAS-103 administered on various schedules against lung metastasis of K1735M2 melanoma. K1735 M2 melanoma cells ($2\times10^5$) were implanted i.v. in B6C3F1 mice on day 0. TAS-103 was administered i.v. from day 1 on the schedules of qdx5 (△), qdx1 (▲), q4dx3 (○), q7dx3 (●) and q10dx2 (□). The ILS% compared to the mean survival in the untreated control group and maximal rate of body weight reduction (Δ $BW_{max}$, g) by TAS-103 were evaluated. The number of animals that died due to toxicity of TAS-103 per group is given in parenthesis.

these 7 tumors. When antitumor activity exceeding 50% was regarded as effective, TAS-103 was effective against all 7 tumors. CPT-11 was effective against 6 of the 7 tumors, with an activity similar to that of TAS-103. CDDP was effective against only 2 of the 7 tumors. VP-16 had no antitumor effect exceeding 50%.

Table IV. Antitumor Effect of TAS-103 against Experimental Lung Metastases of Murine Tumors[a]

| Tumors | Treatment | Schedule | Dose (mg/kg/day) | Pulmonary metastases | | IR (%) | Δ $BW_{max}$ (g)[b] Test/Control |
|---|---|---|---|---|---|---|---|
| | | | | Range | Mean±SD | | |
| B16-BL6 | Control | — | 0 | 243–328 | 289±30 | | |
| | TAS-103 | q7dx3 | 15 | 140–231 | 175±39*** | 39 | +0.3/+0.2 |
| | | | 26 | 105–172 | 126±22*** | 56 | +0.3/+0.2 |
| | | | 45 | 30–84 | 51±19*** | 82 | −2.6/+1.5 |
| | CPT-11 | q4dx3 | 50 | 231–374 | 313±45 | −8.5 | +0.4/+0.2 |
| | VP-16 | qdx5 | 10 | — | — | d.d.[c] | — |
| | CDDP | qdx1 | 7 | 49–118 | 85±25*** | 70 | −0.4/+0.2 |
| UV2237M | Control | — | 0 | 76–202 | 140±46 | | |
| | TAS-103 | q7dx3 | 15 | 9–118 | 56±40** | 60 | 0/−0.2 |
| | | | 26 | 5–29 | 15±10** | 89 | −0.3/−0.2 |
| | | | 45 | 0–7 | 2±3** | 99 | −0.2/−0.2 |
| | CPT-11 | q4dx3 | 50 | 3–152 | 64±56* | 54 | +0.2/−0.2 |
| | VP-16 | qdx5 | 10 | 1–164 | 43±66* | 70 | 0/−0.2 |
| | CDDP | qdx1 | 7 | 1–102 | 48±45** | 66 | −0.5/−0.2 |

*a*) B16-BL6 melanoma or UV2237M fibrosarcoma cells were implanted in syngeneic mice through the tail vein ($1\times10^5$ cells) on day 0. The test agents were administered i.v. from day 1 and 7, respectively. The mice were killed 4 weeks after implantation. The number of pulmonary metastases was counted. The antitumor activity was evaluated as the inhibition rate (IR%) compared to the mean number of pulmonary metastases of the untreated control group.
*b*) Maximal rate of body weight reduction (Δ $BW_{max}$, g) in the drug-treated group is shown in comparison with Δ BW in untreated control mice on the same day.
*c*) Drug deaths.
* $P<0.05$, ** $P<0.01$, *** $P<0.001$ by two-tailed Wilcoxon's exact test as compared to the control group.

## DISCUSSION

Various antitumor drugs target topo I or II, but with different modes of inhibition. For example, camptothecin derivatives and VP-16 have affinity for the complex of topo I or II and DNA, and inhibit the function of topo I or II by stabilizing the complex.[35] Bis(2,6-dioxopiperazine) derivatives such as MST-26 and ICRF-193 act on topo II and inhibit its action without stabilizing the cleavable complex.[36] Intercalators with high affinity for DNA, such as doxorubicin, aclarubicin and mAMSA, also inhibit topo II.[37–39] Doxorubicin and mAMSA, but not aclarubicin, stabilize the topo II-DNA complex. Which mechanism and target in the topo catalytic cycle contribute most to the antitumor effects remains to be clarified. In developing TAS-103, we screened compounds with activity for the relaxation of supercoiled DNA or decatenation of catenated DNA as indices of topo I or II activity. We selected TAS-103 because it inhibited both topo I and II at a low concentration ($10^{-6}$ *M* order), and was as potent as, or more potent than, previous agents. We then examined the mechanisms by which TAS-103 inhibits topo I and II using human tumor cells. TAS-103 cytologically stabilized both topo I- and II-DNA cleavable complexes. However, while the amount of topo II-DNA complex was similar to that formed by VP-16, that of topo I-DNA complex was less than that formed by CPT. The reason for the difference in the topo I- and II-DNA complex formation by TAS-103 remains to be elucidated.

TAS-103 showed marked efficacy against various lung metastatic tumors, and had a broad antitumor spectrum against various human tumors (derived from lung, colon, stomach, and breast cancer) implanted s.c. into nude mice. Concerning the appropriate administration schedule of TAS-103, the greatest efficacy, as well as the widest range of effectiveness, was observed after intermittent administration in both s.c.-implanted Colon 26 and K1735M2 lung metastatic tumors. After consecutive administration, marked body weight loss was observed at a high-dose range, while leukopenia was observed with all administration schedules. The leukopenia appeared to be the dose-limiting toxicity (DLT). With respect to the interval for intermittent administration, the 7-day interval was more effective than the 4- or 10-day interval. This period was consistent with the recovery time of leukocytes, and the optimal administration interval may be strongly associated with recovery from leukopenia.

CPT-11, a topo I inhibitor first approved in Japan, has been reported to cause severe side effects such as diarrhea, as well as leukopenia.[40] Agents inhibiting topo I and II may have higher efficacy than those inhibiting only one of them, but may cause more serious toxicity. To date, RP-60475 is the only topo I and II inhibitor to have

Table V. Efficacy of TAS-103 against Human Tumor Xenografts in Nude Mice[a)]

| Tumors | Origin | Antitumor activity (TWI%) | | | |
|---|---|---|---|---|---|
| | | TAS-103 (q7dx3) 45 mg/kg | CPT-11 (q4dx3) 50 mg/kg | VP-16 (qdx5) 7 mg/kg | CDDP (qdx1) 7 mg/kg |
| Lu-99 | NSCLC | 76** | 65** | 35 | 59** |
| LC-6 | NSCLC | 67*** | 42* | 8 | 44** |
| LX-1 | NSCLC | 78*** | 78*** | 28 (3/7)[b)] | 31** |
| MKN-45 | Gastric ca. | 52** | 56** | 9 | 19 |
| COL-5 | Colon ca. | 60*** | 52*** | 4 | 8 |
| MX-1 | Mammary ca. | 84*** | 96*** | 35 (1/7)[b)] | 83*** |
| PAN-6 | Pancreatic ca. | 65** | 51* | 7 (1/7)[b)] | 27 |
| Results | Effective[c)] (more than 50%) | 7/7 (100%) | 6/7 (86%) | 0/7 (0%) | 2/7 (29%) |

*a*) BALB/c nude mice with s.c.-implanted human tumors with a volume between 50 and 300 $mm^3$ were divided into experimental groups on day 0. The mice were treated i.v. with the test agents from day 0. Experiments were terminated on day 28. Mice were killed, and the tumors excised and weighed. The antitumor activity was evaluated as %TWI relative to the mean tumor weight of the untreated control group. The maximal rate of body weight reduction in the drug-treated group was within 15% of the original body weight of the mice.
*b*) Drug deaths.
*c*) Cases in which antitumor activity exceeded more than 50% of TWI were rated as effective.
* $P<0.05$, ** $P<0.01$, *** $P<0.001$ by two-tailed Welch's $t$ test as compared to the control group.

been tested in clinical trials.[41)] Hepatotoxicity was the DLT, and bone marrow suppression, alopecia and angialgia have been observed. In preliminary toxicity studies with TAS-103 in rats and cynomolgus monkeys, the DLT of TAS-103 was leukopenia, and other toxicity to major organs was not observed.[42)]

TAS-103 is a promising new quinoline derivative which has a unique action, with strong inhibitory effects on the growth of various mouse and human solid tumors *in vivo*, as well as high antitumor activity against lung metastatic cancer. Superior antitumor activities of TAS-103 to those of CPT-11 or VP-16 may be due to dual inhibition of topo I and II. A TAS-103 Phase I study has been initiated in the USA.

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