Antitumor Activity of a Novel Podophyllotoxin Derivative (TOP-53) against Lung Cancer and Lung Metastatic Cancer

Teruhiro Utugi, Jiro Shibata, Yoshikazu Sugimoto, Kumio Aoyagi, Konstanty Wierzbka, Takashi Kobunai, Tadafumi Terada, Tomoko Oh-hara, Takashi Tsuruo, and Yuji Yamada

ABSTRACT

We synthesized a potent new antitumor podophyllotoxin derivative (4β-aminomethyl-4'-O-demethyl-4-deoxyopodophyllotoxin; TOP-53) in our search for a drug that has strong activity against lung cancer and lung metastatic cancer. TOP-53 exhibited twice the inhibitory activity of etoposide (VP-16) against topoisomerase II and induced DNA strand breaks but showed no inhibitory activity against tubulin polymerization. The in vitro cytotoxic activity of TOP-53 assessed as IC50 was 0.016–0.37 μg/ml and 0.26–8.9 μg/ml against murine tumor and human non-small cell lung cancer (NSCLC) cell lines, respectively. TOP-53 exerted significant efficacy equivalent to that of VP-16 on s.c.-implanted murine solid tumors (Colon 26, B16-BL6, and Lewis lung carcinoma) at doses 3-5 times lower than that of VP-16. In human tumor xenografts using NSCLC, TOP-53 was active for four of five tumors, whereas VP-16 was active for two of five tumors. Potent inhibitory activity of TOP-53 was also found against a lung tumor (Lewis lung carcinoma) and four lung metastatic tumors (NL-17 and NL-22 colon cancer, UV2237M fibrosarcoma, and K1735M2 melanoma). TOP-53 appeared to be more active against four of them than VP-16. Thus, TOP-53 is not only active against s.c.-implanted lung cancers but also strongly active against lung localized tumor and metastatic tumors in the lungs. The high selectivity of TOP-53 was attributed to its high potency, distribution, and metabolism of VP-16 and the properties of TOP-53.

INTRODUCTION

VP-16, a podophyllotoxin derivative, is being widely used in cancer chemotherapy, such as for SCLC and malignant lymphoma (1–6). The antitumor activity of VP-16 is associated with its ability to inhibit DNA topo II, unlike the inhibitory activity of its parent compound against tubulin polymerization (7–9). Although VP-16 showed a marked response rate (40–80%) against SCLC (1–4, 6), that rate against NSCLC was very low (10%; Refs. 2, 10, and 11). Therefore, the derivations of VP-16 has continued to allow us to obtain a compound with higher antitumor activity against NSCLC and various lung metastatic tumors in the clinical field.

MATERIALS AND METHODS

Drugs. TOP-53 (M, 585,520) was synthesized by Taiho Pharmaceutical Co., Ltd., (Tokyo, Japan) as described previously (18). VP-16 (M, 588,570) was obtained from Nippon Kayaku Co., Ltd., (Tokyo, Japan). For in vivo experiments, TOP-53 and VP-16 were dissolved in saline and saline containing 6.5% Tween 8 and 3.5% DMSO, respectively. Kinetoplast DNA was obtained from TopoGen, Inc. (Columbus, OH).

Animals. Specific pathogen-free male CDF1, DBA2, BDF1, C57BL/6, BALB/c, B6C3F1, C3H/HeN, and BALB/c nu/nu mice were obtained commercially. Mice were maintained under pathogen-free conditions and we age matched at 7–12 weeks of age at the onset of each experiment.

Tumor Cells. The following murine tumor cells were used: P388 leukemia, LLC, B16-BL6 melanoma, Colon 26 adenocarcinoma, Colon 26 NL-17 and NL-22 sublines, UV2237M fibrosarcoma, and K1735M2 melanoma cells. UV2237M and K1735M2 cells were kindly supplied by Dr. Fidler (University of Texas M. D. Anderson Cancer Center, Houston, TX). NL-17 and NL-22 cells, which produce experimental metastases and spontaneous metastases in the lungs, respectively, were established from the Colon 26 parent cell line (19). For human tumors, the following NSCLC cells were used: Lu-99, LX-1, PC-14, and PC-9. All cell lines were passaged and maintained in vitro in RPMI 1640 containing 10% FCS in a humidified atmosphere of 5% CO2 in air. For in vivo experiments, P388, LLC, B16-BL6, and Colon 26 cells were maintained in the inbred mouse strain of tumor origin. Human NSCLC (Lu-99, LC-11, LC-14, LC-1, and LC-6) were maintained in BALB/c nu/nu mice.

Preparation of Crude Nuclear Extracts and topo II Catalytic Activity Assay. Crude nuclear extracts containing topo II were prepared as described previously (18, 20–22). topo II catalytic activity was measured using the decatenation assay (18, 20, 21). The standard reaction mixture was 50 mM Tris-HCl (pH 7.5), 8.5 mM KCl, 10 mM MgCl2, 0.5 mM DTT, 0.5 mM EDTA, BSA (0.03 mg/ml), and 1 mM ATP. Kinetoplast DNA was decatenated by incubating 4 μl of nuclear extract (0.05 μg of protein) with 1 μg of kinetoplast DNA in the standard reaction mixture for 30 min at 30°C. Reactions were terminated with 5 μl of 5% SDS containing 0.13% bromophenol blue and 50% glycerol. Samples were then electrophoresed in 1% agarose with 40 mM Tris, 2 mM EDTA, and 19 mM acetic acid (pH 8.1) at 50 V for 1 h. Gels were stained with a lower labeling index (16); and (c) a lower amount and activity of topo II (17). Therefore, the low sensitivity of VP-16 to NSCLC could be due to insufficient inhibition of topo II and/or poor distribution in the lungs. This finding suggested that a compound overcoming the following factors should be more effective against NSCLC: (a) a more potent inhibitor of topo II; and (b) a higher concentration and longer distribution of the active form of the compound to the lungs. We attempted to synthesize compounds that are more stable metabolically and have more potential inhibition of topo II and more potent cytotoxicity against NSCLC than VP-16. We finally found a novel podophyllotoxin derivative, TOP-53, that is a non-glycoside, non-ether podophyllotoxin derivative in which an amino alkyl residue was introduced to position 4β of podophyllotoxin, unlike VP-16 (Fig. 1). It is highly water soluble.

In this study, we examined the inhibitory activity of TOP-53 against topo II, and high antitumor activities of TOP-53 against s.c.-implanted solid murine and human tumors in comparison with VP-16. The potent inhibitory activity of TOP-53 against lung metastatic cancer is also discussed in terms of its lung distribution.
Fig. 1. Structure of TOP-53.

with ethidium bromide (1.0 µg/ml) for 30 min and destained for 1 h in H2O. DNA bands were visualized by UV transillumination and photographed using Polaroid type 665 positive/negative film. Inhibitory activity was calculated from densitometrically scanning gel negative. The IC50 was defined as the drug concentration needed to produce a 50% reduction in the amount of minicircle DNA relative to control.

Measurement of DNA Strand Breaks. DNA strand breaks were measured by the fluorometric analysis of DNA unwinding as described previously (23). PC-9 cells were treated with various concentrations of TOP-53 or VP-16 for 3 h. The cells were suspended in 10 mM sodium phosphate buffer (pH 7.2) containing 0.25 mM inositol and 1 mM MgCl2 and then lysed by the addition of 9 mM urea, 2.5 mM diaminocyclohexanetetraacetate, and 0.1% SDS. The samples were incubated in 0.2 n M NaOH (final pH, 12.8) at 15°C for 60 min and then chilled at 0°C, followed by the addition of 1 mM glucose and 14 mM 2-mercaptoethanol. Ethidium bromide, 6.7 µg/ml in 13.3 mM NaOH, was then added to each sample. The relative intensity of fluorescence was measured by spectrophotofluorometry (excitation at 520 nm and detection at 590 nm). The ED50 was defined as the drug concentration needed to produce a 50% reduction of DNA strand breaks relative to the control.

Tubulin Preparation and Antimicrotubular Activity Test. Bovine brain tubulin was prepared as described previously (18–20). Purification proceeded in a buffer composed of 100 mM of 2-(N-morpholino)-ethanesulfonic acid, 1 mM EGTA, 1 mM MgSO4, 5 mM NaH2PO4, and 0.02% NaN3, pH 6.75 (MEM buffer). After one cycle of polymerization-depolymerization, the pellets were washed twice with ice-cold PBS and injected into C3H/HeN mice for 15 min at 37°C. For assembly measurements, turbidity was monitored at 350 nm with a temperature-controlled Hitachi U3210 spectrophotometer. The IC50 was defined as the drug concentration needed to produce a 50% reduction of polymerization relative to the control.

In Vitro Antitumor Activity Evaluation. Tumor cells were plated at the density of 2–3 ¥ 105/mm2 well of flat-bottomed Microtest III plates (Falcon Plastics, Oxnard, CA) and cultured overnight. The cells were treated with various concentrations of drugs for 4 h. After incubation, the cultures were washed with fresh medium to remove the drugs and incubated for 4 or 5 days. The cells were fixed with glutaraldehyde for 15 min and washed three times with water. The cells were stained with 100 µl of 0.05% crystal violet for 1 h, washed twice with ice-cold PBS, and injected into C3H/HeN mice for 15 min at 37°C. For assembly measurements, turbidity was monitored at 350 nm with a temperature-controlled Hitachi U3210 spectrophotometer. The IC50 was defined as the drug concentration needed to produce a 50% reduction of absorbance relative to the control.

In Vivo Antitumor Activity against I.v.-implanted Tumor Cells. P388 leukemia passaged in DBA2 mice was injected i.v. into CDF1 mice (1 ¥ 106 cells/mouse, 7 mice/group). TOP-53 or VP-16 was administered i.v. according to various schedules. Animal mortality was checked daily, and autopsies were performed on dead animals to determine toxic death. The antitumor activity was evaluated as L50% compared to the mean survival of the untreated control group. The mice that had survived for more than 60 days were considered as cured animals.

In Vivo Antitumor Activity against s.c.-growing Tumors. LLC, Colon 26, and B16-BL6 murine tumors were passaged in the inbred mouse strain of tumor origin. The mice were transplanted s.c. with a 2 ¥ 2 ¥ 2-mm3 tumor fragment of LLC or Colon 26 or 5 ¥ 106 cells of B16-BL6 (at least 6 mice/group). TOP-53 and VP-16 were administered i.v. according to various schedules. The mice were weighed twice a week to monitor toxic effects. The mice were killed on day 15, and the tumors were removed and weighed. The antitumor activity was evaluated as the percentage of TGI compared to the mean tumor weight of the untreated control group.

In Vivo Antitumor Activity against Human Tumor Xenografts. BALB/c nu/nu mice were inoculated s.c. with a 3 ¥ 3 ¥ 3-mm3 tumor fragment of human NSCLC (Lu-99, LC-11, PC-14, LX-1, or LC-6). When the tumor mass in the mice had grown between 50 and 300 mm3, the mice were divided into experimental groups consisting of at least six mice/group (day 0). TOP-53 and VP-16 were administered i.v. according to various schedules from day 1. The mice were weighed twice a week to monitor the toxic effects. The tumor mass was measured twice a week up to days 14 to 35. The tumor volume (TV) was calculated according to the following formula:

\[ TV = \left( L \text{ (mm)} \times W^2 \text{ (mm)}^2 \right) / 2 \]

where L and W represent the length and the width of the tumor mass, respectively. Relative tumor volume (RTV) was then calculated as the ratio of TV on day n to that on day 0, according to the following formula:

\[ \%RTV = \frac{TV \text{ on day } n}{TV \text{ on day } 0} \times 100 \]

The inhibition rate of tumor volume (IRTV) on the basis of RTV was then calculated according to the following formula:

\[ \%IRTV = \left( 1 - \frac{\text{Mean RTV of treated group}}{\text{Mean RTV of control group}} \right) \times 100 \]

The largest value for IRTV was designated as IRTVmax, which indicates the greatest effect of each compound.

The rate of body weight reduction (ΔBW) was calculated according to the following formula:

\[ \%\Delta BW = \left( 1 - \frac{\text{Mean BW on day } n}{\text{Mean BW on day } 0} \right) \times 100 \]

The maximal value for ΔBW was designated as ΔBWmax, from which the toxicity of each compound to mice was evaluated.

Experimental Metastasis of LLC, UV2237M, K1735M2, and NL-17. LLCs passaged in C57BL/6 mice were injected into the mice (five or seven mice/group) through the tail vein (3 ¥ 106 cells/mouse). TOP-53 or VP-16 was administered i.v. according to various schedules. The antitumor activity was evaluated as a percentage of surviving mice until day 60.

UV2237M, K1735M2, and NL-17 cells maintained in vitro in RPMI 1640 containing 10% FBS were harvested by brief trypsinization. The cells were washed twice with ice-cold PBS and injected into C3H/HeN mice for UV2237M (1–2 ¥ 106 cells/mouse), B6C3F1 mice for K1735M2 (2 ¥ 106 cells/mouse), or BALB/c mice for NL-17 (2 ¥ 106 cells/mouse) through the tail vein. TOP-53 or VP-16 was administered i.v. according to various schedules. Three or 4 weeks after inoculation, the lungs were excised from the mice and fixed with 10% formalin or Bouin’s solution. The number of pulmonary metastases was counted under a dissecting microscope. The antitumor activity was calculated as a percentage of inhibition rate (IR) compared to the mean number of pulmonary metastases of the control group.

Spontaneous Metastasis of NL-22. NL-22 cells harvested from culture flasks were injected into the footpad of BALB/c mice (1 ¥ 106 cells/mouse). TOP-53 or VP-16 was administered i.v. Two weeks after inoculation, the footpads were amputated. The lungs were excised from the mice on day 32 or 36 and fixed. The antitumor activity was determined as a percentage of IR described above.

Measurement of Tissue Distribution of TOP-53 and VP-16. TOP-53 or VP-16 was injected i.v. into normal and B16 tumor-bearing C57BL/6 mice. The normal lung tissues, lung tumors, or s.c. tumors of mice were collected at various intervals after injection. They were homogenized with a 4 ¥ volume of acetonitrile. After centrifugation, the supernatant was separated and dried up. The dried residue was dissolved in 150 µl of methanol and analyzed by HPLC.
RESULTS

Inhibition of topo II and Induction of DNA Strand Breaks by TOP-53. Inhibitory activity of TOP-53 against topo II was determined as 50% inhibitory concentrations (IC50) of the decatenation of kinetoplast DNA by topo II. The IC50s of TOP-53 and VP-16 were 59 μM and 106 μM, respectively. According to the concentration ratio, TOP-53 exhibited twice the inhibitory activity of VP-16 (Table 1). Furthermore, TOP-53 and VP-16 induced DNA strand breaks with ED50 at 4 and 29 μM, respectively. TOP-53 induced DNA strand breaks at a lower concentration than VP-16. Inhibitory activity of TOP-53 against tubulin polymerization was also determined, and TOP-53 did not show any inhibitory activity at all against tubulin polymerization, similar to VP-16.

In Vitro Cytotoxic Activity of TOP-53. The in vitro cytotoxic activities of TOP-53 and VP-16 were assayed against various murine tumor cells and human NSCLC cells. The tumor cells were treated with various concentrations of the drug for 4 h. After the drug was removed by washing, the cells were incubated in fresh medium containing no drug for 3 days. TOP-53 was cytotoxic to not only murine tumor cells but also human NSCLC cells and was generally more potent than VP-16 (Table 2). The IC50 of TOP-53 were 0.016–0.37 μg/ml for the murine tumor cells and 0.26–8.9 μg/ml for the human NSCLC cells, respectively.

Antitumor Activity of TOP-53 against Experimental Tumors. The in vivo antitumor activity of TOP-53 against murine leukemia cells and solid tumors was determined. P388 murine leukemia cells were implanted i.v. at 1 × 106 cells. LLC or B16-BL6 murine solid tumors were implanted s.c. in 2 × 2 × 2-mm3 fragments or 2 × 105 cells to individual inbred strains of mice. TOP-53 and VP-16 were administered at the optimal dose for each agent from the tail vein with qdx1, consecutive qdx5, or intermittent qdx3 schedule. Antitumor activity against P388 was determined as 50% inhibitory concentrations (IC50) of the decatenation of kinetoplast DNA by topo II. The IC50s of TOP-53 and VP-16 were 59 μM and 106 μM, respectively. According to the concentration ratio, TOP-53 exhibited twice the inhibitory activity of VP-16 (Table 1). Furthermore, TOP-53 and VP-16 induced DNA strand breaks with ED50 at 4 and 29 μM, respectively. TOP-53 induced DNA strand breaks at a lower concentration than VP-16. Inhibitory activity of TOP-53 against tubulin polymerization was also determined, and TOP-53 did not show any inhibitory activity at all against tubulin polymerization, similar to VP-16.

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Antitumor Activity of TOP-53 against Experimental Tumors. The in vivo antitumor activity of TOP-53 against murine leukemia cells and solid tumors was determined. P388 murine leukemia cells were implanted i.v. at 1 × 106 cells. LLC or B16-BL6 murine solid tumors were implanted s.c. in 2 × 2 × 2-mm3 fragments or 2 × 105 cells to individual inbred strains of mice. TOP-53 and VP-16 were administered at the optimal dose for each agent from the tail vein with a single qdx1, consecutive qdx5, or intermittent qdx3 schedule. Antitumor activity against P388 was determined as ILS%, and the activities against LLC and B16-BL6 were determined as %TGI (Table 3). The IC50 of TOP-53 were 0.016–0.37 μg/ml for the murine tumor cells and 0.26–8.9 μg/ml for the human NSCLC cells, respectively.

Table 1 Inhibition of topo II and tubulin polymerization and induction of DNA strand breaks by TOP-53 or VP-16

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC50 (μM)</th>
<th>ED50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOP-53</td>
<td>59</td>
<td>&gt;99</td>
</tr>
<tr>
<td>VP-16</td>
<td>106</td>
<td>NT</td>
</tr>
<tr>
<td>Podophyllotoxin</td>
<td>NT</td>
<td>2.0</td>
</tr>
</tbody>
</table>

a Kinetoplast DNA decatenation assay.
b Anti-microtubular activity using bovine brain tubulin.
c Fluorometric analysis of DNA unwinding using PC-7 human NSCLC cells.
d NT, not tested.

Table 2 In vitro antitumor activity of TOP-53 and VP-16 against various tumor cells

<table>
<thead>
<tr>
<th>Tumors</th>
<th>TOP-53 (μg/ml)</th>
<th>VP-16 (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse tumor cells</td>
<td>0.024</td>
<td>0.092</td>
</tr>
<tr>
<td>LLC</td>
<td>0.029</td>
<td>0.16</td>
</tr>
<tr>
<td>B16</td>
<td>0.37</td>
<td>3.0</td>
</tr>
<tr>
<td>Colon 26</td>
<td>0.016</td>
<td>0.12</td>
</tr>
<tr>
<td>Human tumor cells</td>
<td>0.64</td>
<td>4.9</td>
</tr>
<tr>
<td>Lu-99</td>
<td>0.26</td>
<td>2.1</td>
</tr>
<tr>
<td>PC-14</td>
<td>8.9</td>
<td>48</td>
</tr>
</tbody>
</table>

a Tumor cells were treated with various concentrations of TOP-53 or VP-16 for 4 h. The cells were washed twice with fresh medium and incubated for 3 days. After incubation, the cells were fixed with glutaraldehyde. The cytotoxicity (IC50) was determined by crystal violet dye exclusion assay.
The antitumor activity of TOP-53 against lung metastasis was examined in UV2237M fibrosarcoma and K1735M2 melanoma (Table 5). TOP-53 and VP-16 were administered in three doses at their optimal doses with qdx3 (days 3, 7, and 11) schedule. TOP-53 exhibited inhibited activity of almost 100% on metastatic nodule formation of K1735M2 at high doses.

As shown above, TOP-53 exhibited high antitumor activity equivalent to that of VP-16 against the s.c. transplanted tumor of murine solid carcinoma, whereas it showed extremely high selectivity against lung cancer orthotopically located in the lung and lung metastatic tumors of various carcinomas (colorectal cancer, fibrosarcoma, and melanoma), suggesting that TOP-53 exerts potent antitumor activity against these tumors, and is superior to VP-16.

TOP-53 Distribution in Tissue. We examined the distribution of TOP-53 in the tissue to clarify the reasons for its high selectivity against lung cancer and lung metastatic cancer. As shown in Table 6, TOP-53 was found to be highly distributed in lung tumors and normal lung tissue and to be present at all times. On the other hand, VP-16 distribution in the lungs was low.

DISCUSSION

We have attempted to develop a drug that could be expected to exhibit high antitumor activity against lung cancer and lung metastatic cancer. As a result, we succeeded in synthesizing TOP-53, which has extremely high activity. TOP-53 showed twice the inhibitory activity of VP-16 against topo II and exerted more antitumor activity than VP-16 in vivo against various murine solid carcinomas and human NSCLC. Furthermore, consistent with the concept behind its development, TOP-53 showed extremely high selectivity against lung cancer and lung metastatic cancers (colorectal cancer, fibrosarcoma, and melanoma). TOP-53 thus exhibited potent activity superior to that of VP-16.

We aimed at developing a novel podophyllotoxin derivative that would be highly effective for lung cancer and lung metastatic cancers by using the following strategy. Although VP-16 exerts potent efficacy against SCLC when administered alone (1–6) or in combined use with cis-diamminedichloroplatinum(II) (27), its efficacy for NSCLC is weak (2, 10, 11). We regarded the difference between the activity of VP-16 against NSCLC and SCLC as being attributable to its activity, distribution, and metabolism and the difference between the biological properties of NSCLC and those of SCLC. We considered that a drug that could take these factors into account would be expected to exert efficacy against NSCLC as well. The antitumor activity of VP-16 reaches the maximum when two factors, drug concentration and duration, are satisfied (6, 12). However, the transfer of VP-16 to tumor tissue is approximately equal to the level in blood (13–15), and this level is considered insufficient for high antitumor activity. On the other hand, topo II, i.e., the target enzyme of VP-16, reaches the maximum in phase G2-M of the cell cycle, and is known to be present in a large quantity in rapidly growing cells. The doubling time of NSCLC is about 90–180 days, which is 2–3 times longer than the 55 days for SCLC (16). The topo II level and activity in NSCLC are low, about one-half of those in SCLC (17). The difference in biological properties between NSCLC and SCLC was considered one cause of the difference in the effectiveness of VP-16 against these carcinomas. Therefore, to develop a drug that would be highly effective for NSCLC, higher inhibitory activity against topo II, high transfer to tumor, and sustained activity would be required. To obtain

### Table 4 Efficacy of TOP-53 and VP-16 against human NSCLC xenografts in nude mice

<table>
<thead>
<tr>
<th>Tumors</th>
<th>Schedule</th>
<th>Dose (mg/kg/day)</th>
<th>iRTV&lt;sub&gt;max&lt;/sub&gt; (%)</th>
<th>ΔBW&lt;sub&gt;max&lt;/sub&gt; (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Dose (mg/kg/day)</th>
<th>iRTV&lt;sub&gt;max&lt;/sub&gt; (%)</th>
<th>ΔBW&lt;sub&gt;max&lt;/sub&gt; (%)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lu-99</td>
<td>qdx3</td>
<td>8.0</td>
<td>74</td>
<td>-13.5/+13.2</td>
<td>45.0</td>
<td>66</td>
<td>-13.2/+13.2</td>
</tr>
<tr>
<td>LC-11</td>
<td>qdx3</td>
<td>8.0</td>
<td>59</td>
<td>+0.8/+12.1</td>
<td>45.0</td>
<td>24</td>
<td>-0.4/+10.2</td>
</tr>
<tr>
<td>PC-14</td>
<td>qdx3</td>
<td>10.0</td>
<td>68</td>
<td>-12.4/+6.1</td>
<td>45.0</td>
<td>69</td>
<td>-16.9/+6.1</td>
</tr>
<tr>
<td>LX-1</td>
<td>qdx5</td>
<td>2.3</td>
<td>51</td>
<td>-20.7/-6.8</td>
<td>10.0</td>
<td>49</td>
<td>-20.9/-4.7</td>
</tr>
<tr>
<td>LC-6</td>
<td>qdx5</td>
<td>3.0</td>
<td>35</td>
<td>-7.5/-0.8</td>
<td>34.6</td>
<td>38</td>
<td>-4.70</td>
</tr>
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<td></td>
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</tbody>
</table>

<sup>a</sup> BALB/c nude mice with s.c.-implanted human NSCLC were divided into experimental groups on day 0, when the tumor mass had grown between 50 and 300 mm<sup>3</sup>. The mice were treated i.v. with TOP-53 or VP-16 at qdx3 or qdx5 from day 1. The tumor mass was monitored up to days 14 to 35. The antitumor activity was determined as iRTV<sub>max</sub> (%) in the comparison with untreated control group as described in "Materials and Methods."
TOP-53 was consistent with the concept of development and exhibited extremely high antitumor activity against lung cancer and lung metastatic cancer in various experimental models. We, therefore, examined the reasons for the high selectivity of TOP-53 against lung cancer and lung metastatic cancer. The assessment of metabolic fate in mice and transfer to tissue of cancer-bearing mice revealed that the transfer of TOP-53 to normal lung tissue and tumors in the lungs is higher than that of VP-16 and its duration is longer (Table 6).

Nishimura et al. (30) reported the role of PS in the tissue distribution of basic drugs such as quinidine in rats. Quinidine showed preferential binding to acid phospholipids, especially for PS. On the other hand, PS

a compound with these biological properties, we first examined which would contribute more to antitumor activity, inhibitory activity against tubulin polymerization, or topo II, in the podophyllotoxin skeleton itself. We found that individual inhibitory activity can be separated by introducing a functional group, which differs from the sugar seen in VP-16, to the podophyllotoxin skeleton (20). The correlation between structure and activity indicated that, in the podophyllotoxin skeleton, inhibitory activity against topo II rather than inhibitory activity against tubulin polymerization contributes more to in vivo antitumor activity (18, 20). This is consistent with the facts that VP-16 and teniposide, which show only inhibitory activity against topo II, are commonly used as clinical drugs and that SP-I and SP-G, which have inhibitory activity against tubulin polymerization, are not used because of their severe side effects (28, 29). To achieve more potent inhibitory activity against topo II, the topo II-DNA cleavable complex formation induced by the drug should be increased. Therefore, we introduced a functional group with cationic features to increase the affinity with the polyanion, DNA. Furthermore, as its distribution in organs and metabolic properties were expected to differ from those of VP-16, an aminoalkyl residue, not glucose, was introduced into the position 4β of podophyllotoxin for synthesis of non-glycoside, non-ether 4-desoxypodophyllotoxin derivatives. Among these variously synthesized compounds, compounds without inhibitory activity against tubulin polymerization and with inhibitory activity against topo II were assessed (18, 21). We found TOP-53, which has a unique structure that differs from VP-16 and has high inhibitory activity against topo II.
TOP-53 was considered to show high selectivity against lung cancer and TOP-53 had a high affinity for PS (data not shown). Therefore, the high quinidine in the lungs was suggested due to a high concentration of PS in qdx5. “B16-BL6 tumor metastasized in lung of C57BL/6 mice.

Tumors showed interorgan variation in the tissue distribution, and a large quantity was distributed in the lungs. Therefore, the preferential distribution of quinidine in the lungs was suggested due to a high concentration of PS in the lungs. We have studied the binding of TOP-53 to PS and found that for PS present in the lungs. Thus, TOP-53 was considered to have the

Table 5 Inhibition of experimental lung metastases of murine tumors by treatment with TOP-53 or VP-16

<table>
<thead>
<tr>
<th>Tumors</th>
<th>TOP-53 (mg/kg/day)</th>
<th>VP-16 (mg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose (mg/kg/day)</td>
<td>Range</td>
</tr>
<tr>
<td>UV2237M (fibrosarcoma)</td>
<td>0</td>
<td>230–374</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>12–246</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>108–227</td>
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<tr>
<td></td>
<td>7</td>
<td>8–40–81</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>7–79</td>
</tr>
</tbody>
</table>

ANTITUMOR ACTIVITY OF TOP-53 AGAINST LUNG CANCER

Table 6 Drug distribution in lung after i.v. administration

<table>
<thead>
<tr>
<th>Drug*</th>
<th>Concentration (µg/g tissue)</th>
<th>Tissue</th>
<th>AUC 0–4 h in tissue (µg/g h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOP-53 (4 mg/kg)</td>
<td>15</td>
<td>Normal tissue in lung 18</td>
<td>7.7 ± 0.28</td>
</tr>
<tr>
<td>VP-16 (12 mg/kg)</td>
<td>1.5</td>
<td>Normal tissue in lung 2</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>1.4</td>
<td>Normal tissue in lung 3</td>
<td>0.9</td>
</tr>
</tbody>
</table>

"TOP-53 4 mg/kg and VP-16 12 mg/kg were optimal doses for i.v. administration for qdx5.

REFERENCES

Antitumor Activity of a Novel Podophyllotoxin Derivative (TOP-53) against Lung Cancer and Lung Metastatic Cancer

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