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

Teruhiro Utsugi, Jiro Shibata, Yoshikazu Sugimoto, Kumio Aoyagi, Konstanty Wierzba, 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-desoxypodophyllotoxin; 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-22 and NL-17 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 tissue selectivity and duration. TOP-53 is expected to be highly effective against lung cancer including NSCLC and various lung metastatic tumors in the clinical field.

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 other solid tumors.

To find a more effective compound for NSCLC, we examined the activity, distribution, and metabolism of VP-16 and the properties of NSCLC in comparison with SCLC. The antitumor activity of VP-16 has been considered to become maximum when both drug concentration and duration are adequate (6, 12). However, its distribution to tumor tissue has been reported to be approximately equal to the level in blood (13-15), and this transfer is not satisfactory. On the other hand, NSCLC has the following biological and biochemical properties that differ from SCLC: (a) a 2-3-fold longer doubling time (16); (b) 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 lung tissue. These findings 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 lung tissue. We attempted to synthesize compounds that are more stable metabolically and have more potent 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 4B 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.

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 80 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 were 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, PC-14, LX-1, and LC-6) were maintained s.c. in BALB/c nu/nu mice.

Preparation of Crude Nuclear Extracts and topo II Catalytic Activity

Crude nuclear extracts containing topo II were prepared as described previously (18, 20-22). The purification procedure 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 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 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 ID50 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 M inositol and 1 mM MgCl2 and then lysed by the addition of 9 M urea, 10 mM NaOH, 2.5 mM diaminocyclohexanetetraacetate, and 0.1% SDS. The samples were incubated in 0.2 n NaOH (final pH, 12.8) at 15°C for 60 min and then chilled at 0°C, followed by the addition of 1 M 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 spectrophotometry (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 Antiamicotubular 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 stored at -80°C. Tubulin was polymerized by incubation of 50 µl of tubulin (200 µg protein) with 250 µl of MEM buffer containing 1 mM GTP 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 Vivo Antitumor Activity Evaluation.** Tumor cells were plated at the density of 2–3 × 10^5/38-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 glacial acetic acid for 15 min and washed three times with water. The cells were stained with 100 µl of 0.05% crystal violet solution for 15 min and washed three times with water. Crystal violet was eluted with 0.05 M NaH2PO4/ethanol (1:1 v/v), and the absorbance was measured by an automated spectrophotometric plate reader at a single wavelength of 540 nm. 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 × 10⁴ 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 a percentage of surviving mice until day 60.

**Spontaneous Metastasis of NL-22.** NL-22 cells harvested from culture flasks were injected into the footpad of BALB/c mice (1 × 10⁴ cells/mouse). TOP-53 or VP-16 was administered i.v. according to various schedules. 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.
The antitumor activity of TOP-53 was compared with that of VP-16. The effect of TOP-53 according to the q4dx3 schedule was the highest, followed by that according to qdx5 and qdx1, in that order. The antitumor activity of TOP-53 was compared with that of VP-16. The antitumor activity of TOP-53 administered at the optimal dose was approximately equivalent to that of VP-16 administered at the optimal dose under the same administration schedule in all experimental tumor systems. However, TOP-53 exerted an effect at lower concentrations than VP-16, and its activity was 3–5 times higher than that of VP-16 in the comparison of dose ratio.

**Effect of TOP-53 on Human Tumor Xenografts of NSCLC**. The human tumor xenograft has been reported as one of the best models for predicting drug efficacy in the clinical field (26). The effects of TOP-53 on human tumor xenografts were examined in five human NSCLC/LCCs: Lu-99, LC-11, PC-14, LX-1, and LC-6. TOP-53 and VP-16 were administered at their optimal doses according to the same administration schedules. The effect of VP-16 on life span was 57% according to the qdx5 schedule, 43% to qdx3 schedule, and 0% to qdx2 schedule, and the activities of TOP-53 and VP-16 were compared by the percentage of mice surviving until 60 days after administration (Fig. 2). TOP-53 exhibited a potent inhibitory effect at lower concentrations than VP-16, and its activity was 3–5 times higher than that of VP-16 in the comparison of dose ratio.

**Antitumor Activity of TOP-53 against Lung Metastasis Tumors.** We examined the antitumor activity of TOP-53 against lung tumors and lung metastatic tumors. Lung tumors were developed by i.v. implantation of 1 × 10⁶ LLC cells into the tail vein. TOP-53 or VP-16 was administered with qdx5 (days 1–5), qdx3 (days 1, 5, and 9), or qdx2 (days 4 and 8) schedule, and the activities of TOP-53 and VP-16 were compared by the percentage of mice surviving until 60 days after administration (Fig. 2). TOP-53 exhibited a potent inhibitory effect equivalent to that of VP-16 on s.c. implanted LLC (Table 3). On the other hand, TOP-53 exerted antitumor effects on lung tumors at the highest dose on the qdx5 schedule in 100%, qdx3 in 86%, and qdx2 in 80%. Thus, effectiveness in increasing life span was observed with all administration schedules. The effect of VP-16 on life span was 57% according to the qdx5 schedule, 43% with the qdx3 schedule, and 0% with qdx2, all less effective than TOP-53. TOP-53 exhibited excellent effectiveness at the low dose as well and was superior to VP-16. The effects of TOP-53 on the formation of spontaneous lung metastatic tumor and experimental lung metastatic tumor were examin-
exhibited inhibitory activity as high as 71% against experimental lung metastasis tumor at the highest dose, VP-16 showed high antitumor activities of 91 and 81%, respectively, when TOP-53 was administered to spontaneous lung metastatic tumor of Colon 26 sublines. NL-22 and NL-17 (Fig. 3). TOP-53 and VP-16 were administered in three doses at their individual maximum doses against these tumors, and is superior to VP-16.

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 a qdx3 (days 3, 7, and 11) schedule. TOP-53 exhibited inhibitory activity as high as 71% against experimental lung metastatic tumor of UV2237M at the maximum dose, but the inhibitory activity of VP-16 was only 44%. Both TOP-53 and VP-16 showed potent inhibitory effects 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 carcinomas, 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-diaminedichloroplatinum(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 G$_2$-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>
<thead>
<tr>
<th>Tumors</th>
<th>Schedule</th>
<th>Evaluation</th>
<th>Dose (mg/kg/day)</th>
<th>Efficacy (%)</th>
<th>Dose (mg/kg/day)</th>
<th>Efficacy (%)</th>
<th>TOP-53 vs. VP-16</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLC</td>
<td>qdx3 (days 4-8)</td>
<td>TGI</td>
<td>4</td>
<td>95</td>
<td>12</td>
<td>60</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>B16-BL6 melanoma</td>
<td>qdx3 (days 4-8)</td>
<td>TGI</td>
<td>7</td>
<td>98</td>
<td>36</td>
<td>98</td>
<td>NS</td>
</tr>
<tr>
<td>P388 lymphoma i.v.</td>
<td>qdx3 (days 1-5)</td>
<td>ILS</td>
<td>2.5</td>
<td>35</td>
<td>7</td>
<td>75</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>qdx3 (days 1-5)</td>
<td>ILS</td>
<td>7</td>
<td>81</td>
<td>36</td>
<td>99</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>qdx3 (days 1-5)</td>
<td>ILS</td>
<td>7</td>
<td>236(3/4)x</td>
<td>7</td>
<td>226(3/4)x</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>qdx3 (days 1-5)</td>
<td>ILS</td>
<td>7</td>
<td>243(3/4)x</td>
<td>36</td>
<td>Cure(7/7)x</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>qdx3 (day 1)</td>
<td>ILS</td>
<td>12</td>
<td>103</td>
<td>36</td>
<td>103</td>
<td>NS</td>
</tr>
</tbody>
</table>

* By two-tailed Welch's t test for TGI or Mann-Whitney U test for ILS as compared to control group. NS, not significant.

# Number of cured mice. ILS values were calculated without cured mice.
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 podophyllotoxin skeleton itself. From these 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.

Fig. 2. Antitumor activity of TOP-53 and VP-16 against LLC in lung. LLC were injected into the mice through the tail vein to form lung tumors. TOP-53 or VP-16 was administered i.v. either on a consecutive or intermittent administration schedule. The antitumor activity was evaluated as the percentage of surviving mice at day 60. A, qdx5 (days 1–5); • TOP-53; ○, VP-16. B, qdx3 (days 1, 5, and 9); • TOP-53; ○, VP-16, or qdx2 (days 4 and 8); △ TOP-53; Δ, VP-16.

Fig. 3. Antitumor activity of TOP-53 and VP-16 against s.c.-implanted Colon 26 or spontaneous and experimental metastases of Colon 26 sublines. A, for s.c. tumor, a tumor fragment of Colon 26 was inoculated s.c. into mice. TOP-53 (•) or VP-16 (○) was administered i.v. with qdx5 (days 1–5). The mice were killed on day 15, and the tumors were removed and weighed. The antitumor activity was evaluated as TGI%. B, for spontaneous lung metastasis experiments, NL-22 cells were injected into the footpad of mice. TOP-53 or VP-16 was administered i.v. with qdx5 from day 1 (•, TOP-53; ○, VP-16) or day 17 (△ TOP-53; Δ, VP-16). Two weeks after inoculation, the footpads were amputated. C, for experimental lung metastasis experiments, NL-17 cells were injected into mice through the tail vein. TOP-53 (•) or VP-16 (○) was administered i.v. with qdx5 (days 1–5). The lungs were excised from the mice on day 32 or 36 for NL-22 and day 21 for NL-17 and fixed. The number of pulmonary metastases was counted, and the antitumor activity was evaluated as the percentage of inhibition rate (Inhibition rate [%]).
showed interorgan variation in the tissue distribution, and a large quantity was distributed in the lungs. Therefore, the preferential distribution of quinidine in rats. J. Pharmacobio-Dyn., 10: 135-141, 1987.

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

<table>
<thead>
<tr>
<th>Drug</th>
<th>Tissue</th>
<th>Concentration (μg/g)</th>
<th>AUC 0-4 h in tissue (μg/g · h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOP-53 (4 mg/kg)</td>
<td>Lung tumorb</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Normal tissue in lung</td>
<td>18</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>s.c. tumorb</td>
<td>1.5</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Lung tumorb</td>
<td>0.82</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Normal tissue in lung</td>
<td>2.5</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>s.c. tumorb</td>
<td>1.4</td>
<td>0.39</td>
</tr>
<tr>
<td>VP-16 (12 mg/kg)</td>
<td>Lung tumorb</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>Normal tissue in lung</td>
<td>18</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>s.c. tumorb</td>
<td>1.5</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>Lung tumorb</td>
<td>0.82</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Normal tissue in lung</td>
<td>2.5</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>s.c. tumorb</td>
<td>1.4</td>
<td>0.39</td>
</tr>
</tbody>
</table>

- TOP-53 4 mg/kg and VP-16 12 mg/kg were optimal doses for i.v. administration for quinidine.
- B16-BL6 tumor metastasized in lung of C57BL/6 mice.
- B16-BL6 tumor in s.c. site of C57BL/6 mice.
- ND, not detected.

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