Abstract. In this review, in the search for the development of new anticancer drugs, the effects of compounds isolated from various medicinal plants on tumor growth and metastasis, using mice bearing a highly metastatic drug-resistant mouse tumor, were studied. The antitumor and antimetastatic actions of stilbene derivatives isolated from Polygonum and Cassia species were examined. Among the stilbene derivatives, resveratrol and cassiagrol A (stilbene dimer) displayed antitumor and antimetastatic actions through the inhibition of tumor-induced neovascularization in in vitro and in vivo models. It was found that two chalcone derivatives from Angelica keiskei roots also inhibited tumor growth and metastasis in tumor-bearing mice through the inhibition of tumor-induced neovascularization and/or the inhibition of immune suppression caused by tumors. Recently, basidiomycete fungi have been used for the treatment of cancer. Then, the low molecular weight substances were isolated from Agaricus blazei and Ganoderma lucidum as antitumor and antimetastatic substances. It is suggested that these substances of basidiomycete also inhibited tumor growth and metastasis to the lung through the inhibition of tumor-induced neovascularization and/or the inhibition of immune suppression caused by tumors.

Cancer is the largest single cause of death in both men and women, claiming over 6 million lives each year worldwide. Cancer chemotherapy drugs such as 5-fluorouracil derivatives, cisplatin, mitomycin, adriamycin, taxol, etc., have been used extensively for the treatment of certain types of cancer. However, with these treatments, severe gastrointestinal toxicity, with diarrhea and mucosis and hematological toxicity, with leucopenia and immune suppression, appear to be dose-limiting factors. After the removal of a malignant tumor by surgical operation, radiation therapy and/or adjuvant therapy with cancer chemotherapy drugs may be curative. However, the removal of certain cancers, for example, breast carcinoma, colon carcinoma and osteogenic sarcoma, may be followed by the rapid growth of distant metastases to lung, liver etc. Therefore, it is necessary to develop new anticancer agents with antitumor and antimetastatic activities but without adverse reactions such as gastrointestinal toxicity, myelotoxicity and immune suppression caused by cancer chemotherapeutic drugs.

In this review, the effect of natural products isolated from various medicinal plants on tumor growth and metastasis using mice bearing the highly metastatic, drug-resistant mouse tumor, Lewis lung carcinoma (LLC), was described as follows: 1) antitumor and antimetastatic actions of stilbene derivatives isolated from medicinal plants; 2) antitumor and antimetastatic actions of chalcone derivatives isolated from Angelica keiskei roots; 3) antitumor and antimetastatic actions of active substances isolated from basidiomycete fungus. Furthermore, to clarify the mechanisms of antitumor and antimetastatic actions of natural products, experiments were carried out to examine the effects of natural products on the angiogenesis and immune function in both in vitro and in vivo experiments.

Antitumor and antimetastatic actions of stilbene derivatives isolated from medicinal plants

Stilbenes are naturally occurring phytoalexins found in medicinal plants of Polygonum species and Rheum species (Polygonaceae) and Cassia species (Leguminosae) (1-4). Among the stilbene derivatives, resveratrol (3,4',5-trihydroxystilbene) and resveratrol-3-O-D-glucoside (piceid)
are also found in grapes and red wine. In a previous paper, it was reported that resveratrol, piceid and 2,3,4',5-tetrahydroxystilbene-2-O-D-glucoside isolated from Polygonum species roots reduced the elevation of lipid levels (5), and that 2,3,4',5-tetrahydroxy-2-O-D-glucoside markedly prevented liver damage induced by high lipid peroxidized diets (6). Furthermore, it was reported that resveratrol strongly inhibited the formation of 5-lipoxygenase products, 5-hydroxy-6,8,11,14-eicosatetraenoic acid, leukotrienes B₄ and C₄, and the cyclooxygenase product thromboxane B₂ from arachidonic acid (7-9), and that resveratrol inhibited the arachidonic acid-induced platelet aggregation (7). Moreover, resveratrol and its derivatives have been further shown to strongly inhibit the
degranulation of human polymorphonuclear leukocytes (9).

The antitumor and antimetastatic actions of resveratrol and its glucosides were studied in Lewis lung carcinoma (LLC)-bearing mice (10, 11). Recently, there have been a number of reports that resveratrol inhibits tumor growth and causes apoptosis as a cancer chemopreventive agent (12-19). Although resveratrol is reported to contain a cancer chemopreventive agent, the inhibitory action by resveratrol and its glycoside on distant metastases to other organs from primary solid tumors is as yet unproven. Piceid and 2,3,4',5-tetrahydroxy-2-O-D-glucoside (Figure 1) inhibited tumor

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Metastasis to the lung (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LLC-bearing mice (control)</td>
<td>3/5 (60)</td>
</tr>
<tr>
<td>+2,3,5,4'-Tetrahydroxystilbene-2-O-D-glucoside (50 mg/kg x 2/day)</td>
<td>5/5 (100)</td>
</tr>
<tr>
<td>(150 mg/kg x 2/day)</td>
<td>1/5 (20)</td>
</tr>
<tr>
<td>+ Piceid (100 mg/kg x 2/day)</td>
<td>5/5 (100)</td>
</tr>
<tr>
<td>(300 mg/kg x 2/day)</td>
<td>1/5 (20)</td>
</tr>
</tbody>
</table>

Solid-type LLC was prepared by subcutaneous transplantation into the right hind paw on day 0. 2,3,5,4'-Tetrahydroxystilbene-2-O-D-glucoside or piceid was administered orally twice daily for 32 consecutive days, starting 12 h after tumor implantation. Values are means±S.E. of 5 mice.

Figure 1. Structure of stilbenes isolated from Polygonum species roots.

Figure 2. Effects of 2,3,5,4'-tetrahydroxy-stilbene-2-O-D-glucoside and piceid isolated from Polygonum species roots on metastasis to the lung in LLC-bearing mice.

Table I. Effects of 2,3,5,4'-tetrahydroxy-2-O-D-glucoside and piceid isolated from Polygonum species roots on metastasis to the lung in LLC-bearing mice.

![Chemical structure of stilbenes](image)

![Graphs showing tumor growth in LLC-bearing mice.](image)
growth time-dependently after oral administration of 300 or 150 mg/kg twice daily (Figure 2). Furthermore, the two stilbene glucosides inhibited lung metastasis in LLC-bearing mice (Table I). It has been suggested that piceid or 2,3,4',5'-tetrahydroxystilbene-2-O-D-glucoside administered orally may be converted to an aglycone form of resveratrol (Figure 1) by hydrolysis. Therefore, the effects of resveratrol on tumor

Figure 3. Antitumor and antimetastatic activities of resveratrol in LLC-bearing mice. Solid-type LLC was prepared by subcutaneous transplantation into the back on day 0. Resveratrol was administered intraperitoneally once daily for 21 consecutive days. Values are means ± S.E. of 7 mice.

Figure 4. Effects of resveratrol on [3H]-thymidine incorporation into DNA of LLC cells. Results are expressed as mean±S.E. of four experiments. *Significantly different from [3H]-thymidine alone, p<0.05.

Table II. Effects of resveratrol isolated from Polygonum cuspidatum roots on apoptosis, G0/G1, S- and G2/M-phases of cell cycle in LLC cells.

<table>
<thead>
<tr>
<th>Resveratrol (µM)</th>
<th>Apoptosis</th>
<th>G0/G1</th>
<th>S</th>
<th>G2/M</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12.1±0.36</td>
<td>50.0±1.97</td>
<td>35.2±1.72</td>
<td>14.8±0.29</td>
</tr>
<tr>
<td>5</td>
<td>9.43±0.51</td>
<td>44.6±0.75</td>
<td>37.9±1.25</td>
<td>17.5±0.49</td>
</tr>
<tr>
<td>10</td>
<td>9.65±0.61</td>
<td>39.9±1.54</td>
<td>43.8±2.12</td>
<td>16.3±0.70</td>
</tr>
<tr>
<td>50</td>
<td>12.5±0.97</td>
<td>46.8±1.15</td>
<td>22.1±1.03*</td>
<td>31.1±0.26*</td>
</tr>
<tr>
<td>100</td>
<td>20.6±1.35*</td>
<td>52.9±1.16</td>
<td>29.2±0.27*</td>
<td>17.8±1.42</td>
</tr>
</tbody>
</table>

Values are means±S.E. of three experiments. *Significantly different from medium alone, p<0.05.

growth time-dependently after oral administration of 300 or 150 mg/kg twice daily (Figure 2). Furthermore, the two stilbene glucosides inhibited lung metastasis in LLC-bearing mice (Table I). It has been suggested that piceid or 2,3,4',5'-tetrahydroxy-stilbene-2-O-D-glucoside administered orally may be converted to an aglycone form of resveratrol (Figure 1) by hydrolysis. Therefore, the effects of resveratrol on tumor
growth and lung metastasis were examined in LLC-bearing mice. As shown in Figure 3, final tumor weight was significantly inhibited by intraperitoneally-administered resveratrol at doses of 2.5 and 10 mg/kg. Resveratrol (2.5 and 10 mg/kg) significantly reduced the number of tumor cell colonies that metastasized to the lung compared to untreated LLC-bearing mice (Figure 3).

To clarify the mechanisms of antitumor and antimetastatic actions by resveratrol, the effects of resveratrol on the DNA synthesis of LLC cells were examined. The results showed that resveratrol inhibited the DNA synthesis with an IC₅₀ of 6.8 μM (Figure 4). Resveratrol at 100 μM caused apoptosis, decreased the S-phase population at 50 and 100 μM and increased the G₂/M-phase at 50 μM in LLC cells (Table II). The solid tumors cause neovascularization, and the resultant angiogenesis from solid tumors stimulated growth and metastasis. Resveratrol inhibited tumor-induced neovascularization (in vivo) at doses of 2.5 and 10 mg/kg (Figure 5). In addition, resveratrol at 10, 50 and 100 μM inhibited the Matrigel-induced capillary-like network tube formation of human umbilical vein endothelial cell (HUVEC) (in vitro) (Figure 6). Tumor cells are thought to secrete angiogenic factor(s) that induce neovascularization around the tumors (20-22). Vascular endothelial growth factor (VEGF) is a secretory angiogenic factor (23). Resveratrol inhibited the binding of VEGF to HUVEC at 10 to 100 μM (Figure 7). These findings suggest that the mechanism of antitumor and antimetastatic actions of resveratrol might be attributed to the inhibition of DNA synthesis in LLC cells and the inhibition of tumor-induced neovascularization through the inhibition of capillary-like tube formation from vascular endothelial cells.

The heartwood of Cassia garrettiana Craib (Leguminosae) is a Thai drug called "Sa me sarn". It has been used as a mild cathartic in folk medicine. Although it has recently been proposed that extracts of C. garrettiana heartwood have antitumor activity, the basis for this hearsay is unclear. The stilbene derivatives cassigarol A (picetannol dimer) and piceatannol (3,3',4',5-tetrahydroxystilbene) have been isolated from the heartwood of C. garrettiana (Figure 8). The antitumor and antimetastatic actions of cassigarol A,
Figure 6. a) Photograph of capillary-like network formation from human umbilical vein endothelial cells (HUVEC) in the presence of various concentrations of resveratrol (x 100 magnification). b) Effects of resveratrol on capillary-like tube formation by HUVEC. HUVEC (2 x 10^4 cells) were seeded onto the Matrigel and incubated with the indicated amounts of resveratrol at 37°C for 24 h in a humidified 5% CO₂ atmosphere. Four different fields per well were photographed. The total length of tube structures in each photograph was measured using Adobe Photoshop software. Results are means ± S.E. of four experiments. *Significantly different from medium alone, p<0.05.
Piceatannol and piceatannol acetate were examined in LLC-bearing and in primary tumor-removed mice. As shown in Figure 9, cassigarol A (50 and 100 mg/kg twice daily) significantly inhibited the tumor weight on day 15 compared to the weight in untreated LLC-bearing mice, but piceatannol had no effect. Cassigarol A, piceatannol and piceatannol acetate (50 and 100 mg/kg twice daily) prolonged the survival time and increased the survival rate compared to those in untreated tumor-removed mice (Figure 10). Cassigarol A, piceatannol and piceatannol acetate inhibited the increases of metastasis to the lung (Figure 11). Furthermore, to clarify the antitumor and antimetastatic activities by cassigarol A, piceatannol or piceatannol acetate, the anti-angiogenic actions of the above stilbenes were examined in in vitro experiment. Cassigarol A and piceatannol inhibited the angiogenesis of HUVEC at concentrations of 10 to 100 μM, but piceatannol acetate did not have an effect (Figure 12).

Therefore, it is suggested that the antitumor and/or antimetastatic activities of cassigarol A and piceatannol might be due to the inhibition of angiogenesis induced by tumor. Thus, it is suggested that stilbene compounds (monomer and dimer compounds) isolated from Polygonum and Cassia species have antitumor and antimetastatic actions by the inhibition of tumor-induced angiogenesis in in vitro and in vivo experiments.

Figure 7. Effects of resveratrol on the binding of 125I-vascular endothelial growth factor (VEGF) to HUVEC. Subconfluent HUVEC were incubated with the indicated amounts of resveratrol for 30 min at room temperature. Subsequently, 125I-VEGF (4.26 MBq/μg) was added and incubated for 3 h in a humidified 5% CO₂ atmosphere. After unbound radioligand has been removed, the cells were solubilized in 1% SDS. The amount of 125I-VEGF bound to HUVEC was measured using a gamma counter. Results are means±S.E. of four experiments. ∗Significantly different from medium alone, p<0.05.

Figure 8. Structures of stilbenes isolated from Cassia garrettiana heartwood.

Figure 9. Effects of cassigarol A isolated from Cassia garrettiana heartwood on tumor growth in LLC-bearing mice. Solid-type LLC was prepared by subcutaneous transplantation into the right backs of mice on day 0. Cassigarol A (50 or 100 mg/kg) was administered orally twice daily for 14 days. Values are means±S.E. of 4 to 7 mice. ∗Significantly different from LLC-bearing mice, p<0.05.
Figure 10. Effects of cassigrol A and piceatannol isolated from C. garrettiana heartwood on survival time and survival rate in carcinectomized mice. On day 15, the solid tumor tissues were removed under pentobarbital anesthetic and weighed. Thereafter, cassigrol A or piceatannol was again administered orally twice daily for 17 or 24 days, respectively. The survival time and number of surviving tumor-removed mice were determined.

Figure 11. Effects of cassigrol A and piceatannol on lung metastasis in carcinectomized mice. Values are means ± S.E. of 4 to 7 mice.
Antitumor and antimetastatic actions of chalcone derivatives isolated from *Angelica keiskei* roots

*Angelica keiskei* Koizumi (Asitaba in Japanese), a plant found along the Pacific Coast of Japan, is used as a diuretic, laxative, analeptic and galactagogue. Although it has been proposed that the roots and leaves of *A. keiskei* have preventive effects against coronary heart diseases, hypertension and cancer, there is no clear evidence. It has been reported that chalcone derivatives, such as xanthoangelol and 4-hydroxyderricin (Figure 13), are...
isolated from this root as major components of yellow substances (24, 25). In the series of pharmacological actions of A. keiskei root, xanthoangelol, 4-hydroxyderricin, and xanthoangelol B, E and F inhibited phenylephrine-induced vasoconstriction (28). Recently, it has been reported that a 50% ethanol extract of A. keiskei roots has antitumor and

Table III. Effects of xanthoangelol isolated from Angelica keiskei roots on tumor weight in LLC-bearing mice and on lung and tumor metastasis to the lung of carcinectomized LLC-bearing mice.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tumor weight (mg) of the surgically tumor-removed mice</th>
<th>Lung (mg)</th>
<th>Lung metastasis (number of colonies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0±0</td>
<td>145.1±8.33*</td>
<td>0±0</td>
</tr>
<tr>
<td>LLC-removed mice</td>
<td>1548.0±171.1</td>
<td>211.9±8.80</td>
<td>23±3</td>
</tr>
<tr>
<td>Xanthoangelol (25 mg/kg x 2/day)</td>
<td>906.9±63.0*</td>
<td>182.5±12.0</td>
<td>8±4</td>
</tr>
<tr>
<td>(50 mg/kg x 2/day)</td>
<td>682.3±63.0*</td>
<td>160.2±10.3*</td>
<td>11±8*</td>
</tr>
</tbody>
</table>

Xanthoangelol (25 and 50 mg/kg) was administered orally twice daily for 15 days to LLC-bearing mice. On day 15, solid primary tumor tissues were removed, and then xanthoangelol was again administered until death. On day 8, surviving tumor-removed mice were killed. Values are means±S.E. of 6-13 mice. *Significantly different from LLC-removed mice, p<0.05.

Figure 15. Effects of xanthoangelol on survival time and survival rate in carcinectomized mice. On day 15, the solid primary tumor tissues were removed under pentobarbital anesthesia and weighed. Thereafter, xanthoangelol was again administered orally twice daily for 15 days. The survival time and number of surviving tumor-removed mice were determined. The LLC-bearing group (control) consisted of 13 mice; the xanthoangelol (25 and 50 mg/kg, twice daily)- and CDDP (1.25 mg/kg, i.p.)-treated groups consisted of 6 mice each.

Figure 16. Effects of xanthoangelol on tumor weight (a) and metastasis to the liver (b) in intrasplenic LLC-implanted mice. Values are means±S.E. of 5-13 mice. Sham-operated group (normal) consisted of 5 mice; the untreated LLC-bearing group (control) consisted of 13 mice; xanthoangelol (50 and 100 mg/kg daily)-treated groups consisted of 6 mice each.
antimetastatic actions in LLC-bearing mice, and that xanthoangelol and 4-hydroxyderricin are isolated as antitumor and antimetastatic substances (26, 27). The effects of two chalcones (xanthoangelol and 4-hydroxyderricin) on tumor growth and metastasis to lung or liver using mice with subcutaneously-implanted LLC cells, xanthoangelol (50 mg/kg twice daily) significantly inhibited tumor growth on days 11 and 14 compared to growth in untreated LLC mice (Figure 14). On day 15, tumor tissues were removed and weighed, and it was found that tumor weight was reduced by the twice-daily oral administration of xanthoangelol (25 and 50 mg/kg). Xanthoangelol (50 mg/kg twice daily) inhibited metastasis to the lung in tumor-removed mice and the

Figure 17. Photographs showing inhibition by xanthoangelol of LLC tumor metastasis to the liver (a) and histology showing inhibition of LLC metastatic tumor growth in the liver (b) on day 20 in mice with intrasplenically-implanted LLC.
Figure 18. Photographs showing inhibition of LLC-induced neo-vascularization by xanthoangelol in LLC-packed chamber-bearing mice. Chambers packed with LLC cells were implanted subcutaneously into a dorsal air sac of C57BL/6 mice on day 0. Air sacs of normal mice treated with DMEM alone (normal) and those of mice with LLC-packed chambers without treatment or with i.p. administration of 5, 10 and 20 mg/kg of xanthoangelol from days 1 to 5 are shown.

Figure 19. Light micrographs of Matrigel-induced formation of capillary-like tubes by HUVEC in the presence of various concentrations of xanthoangelol (x 100 magnification).
Figure 20. Effects of xanthoangelol on Matrigel-induced formation of capillary-like tubes by HUVEC. Values are means±S.E. of four experiments. *Significantly different from medium alone, p<0.05.

Figure 21. Effects of xanthoangelol on the binding of $^{125}$I-VEGF to HUVEC. Values are means±S.E. of four experiments. *Significantly different from $^{125}$I-VEGF alone, p<0.05.

Figure 22. Effects of xanthoangelol on $[^3H]$-thymidine incorporation into DNA in LLC cells and HUVEC. Values are ± S.E. of four experiments. *Significantly different from $[^3H]$-thymidine alone, p<0.05.

Figure 23. Effects of 4-hydroxyderricin isolated from Angelica keiskei roots on tumor growth in mice with subcutaneously-implanted LLC. Values are means±S.E. of 4-6 mice.
Figure 24. Effects of 4-hydroxyderricin on tumor weight in mice with subcutaneously-implanted LLC. Values are means±S.E. of 4-6 mice.

Figure 25. Effects of 4-hydroxyderricin on survival time and survival rate in mice after the removal of tumors by surgical operation.

Figure 26. Effects of 4-hydroxyderricin on the lung weight (a) and lung metastasis (b) in mice after the removal of tumors by surgical operation. Values are means±S.E. of 4-6 mice.
<table>
<thead>
<tr>
<th>Cell number (x 10^6 cells/spleen)</th>
<th>Mean±S.E.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>n</strong></td>
<td><strong>Lymphocyte</strong></td>
</tr>
<tr>
<td>Normal LLC-removed mice (control)</td>
<td>4</td>
</tr>
<tr>
<td>Surviving plus dead mice</td>
<td>6</td>
</tr>
<tr>
<td>Surviving mice + 4-Hydroxyderricin^1 (25 mg/kg x 2/day, p.o.)</td>
<td>(0)</td>
</tr>
<tr>
<td>Surviving plus dead mice</td>
<td>6</td>
</tr>
<tr>
<td>(Surviving mice) (50 mg/kg x 2/day, p.o.)</td>
<td>(1)</td>
</tr>
<tr>
<td>Surviving plus dead mice</td>
<td>6</td>
</tr>
<tr>
<td>(Surviving mice) (5 mg/kg x 2/week, i.p.)</td>
<td>(5)</td>
</tr>
<tr>
<td>+ Doxorubicin^2 (5 mg/kg x 2/week, i.p.)</td>
<td>5</td>
</tr>
</tbody>
</table>

^14-Hydroxyderricin (25 or 50 mg/kg) was administered orally twice daily for 15 days to LLC-bearing mice. Doxorubicin (5 mg/kg) was administered intraperitoneally to mice on days 1 and 8 after implantation of tumor cells. On day 15, the solid tumor tissues were removed, and then 4-hydroxyderricin or doxorubicin was again administered until death. On day 16, the surviving tumor-removed mice were killed. The spleen and thymus weights of dead mice and surviving mice killed on day 16 were measured. Values are expressed as mean±S.E. of 4-6 mice.

increase of lung weight that occurred in tumor-removed mice, but at a dose of 25 mg/kg twice-daily it had no effect (Table III). Furthermore, xanthoangelol (50 mg/kg twice daily) prolonged survival time and increased the survival rate compared to untreated tumor-removed mice, but at a dose of 25 mg/kg twice-daily it had no effect (Figure 15).

In mice with intrasplenically-implanted LLC cells, tumor weight in the spleen was not affected by oral administration of xanthoangelol daily at 50 or 100 mg/kg for 19 days. Mice with intrasplenically-implanted LLC had tumor metastasis to the liver, with about 115 tumor colonies per mouse. Xanthoangelol (50 or 100 mg/kg daily) significantly reduced the number of tumor cell colonies that metastasized to the liver compared to the number in mice bearing intrasplenic LLC (Figures 16 and 17). Growth of metastatic tumors in the livers of LLC-bearing mice was also inhibited by orally-administered xanthoangelol at a daily dose of 50 or 100 mg/kg (Figure 17).

To clarify the mechanisms of the antitumor and anti-metastatic actions of xanthoangelol, the anti-angiogenic actions of xanthoangelol were examined in *in vivo* or *in vitro* experiments. Five days after implantation of LLC cells that were packed into a membrane chamber, neovascularization was evident in the region in contact with the chamber containing LLC cells. Intraperitoneally-administered xanthoangelol (10 and 20 mg/kg) prevented the neovascularization induced by LLC cells (Figure 18). Furthermore, HUVECs formed capillary-like networks on Matrigel 24 h after seeding. Xanthoangelol inhibited the angiogenesis of HUVECs at 1 to 100 μM (Figures 19 and 20). Xanthoangelol significantly inhibited the binding of VEGF to HUVECs at 1 to 100 μM (Figure 21). On the other hand, xanthoangelol inhibited DNA synthesis in LLC cells at 10-100 μM, but it had no effect on DNA synthesis in HUVECs (Figure 22).
Figure 27. Effects of 4-hydroxyderricin on intrasplenic tumor growth on day 20 in LLC-bearing mice.

a) Values are means ± S.E. The untreated LLC-bearing group (control) and 4-hydroxyderricin-treated groups (50 and 100 mg/kg, daily) consisted of 8 mice each.

b) Photographs showing inhibition of primary tumor growth in the spleen by 4-hydroxyderricin. c) Histological evidence of the inhibition of intrasplenic tumors (x 400 magnification) by 4-hydroxyderricin in LLC-bearing mice. Tumors, closed arrows; lymphocytes, open arrows.
Therefore, these findings suggest that the antitumor and antimetastatic actions of xanthoangelol might involve inhibition of DNA synthesis in LLC cells and inhibition of tumor-induced neovascularization through inhibition of capillary-like tube formation of vascular endothelial cells and the binding of VEGF to vascular endothelial cells. 4-Hydroxyderricin (25 and 50 mg/kg twice daily) significantly inhibited tumor growth on days 8 to 14 in mice with subcutaneously-implanted LLC (Figure 23). Tumor weight on day 15 was reduced by the twice-daily oral administration of 4-hydroxyderricin (50 mg/kg) (Figure 24). Furthermore, 4-hydroxyderricin (25 and 50 mg/kg twice daily) prolonged the
survival time and increased the survival rate compared to those in mice after the removal of tumors (Figure 25). On the other hand, the intraperitoneal administration of doxorubicin (5 mg/kg x 2/week) inhibited primary tumor growth in untreated LLC-bearing mice, but doxorubicin shortened the survival time and reduced the survival rate compared to those in untreated tumor-removed and 4-hydroxyderricin-treated mice (Figure 25).

4-Hydroxyderricin (25 and 50 mg/kg twice daily) inhibited metastasis to the lung in tumor-removed mice and the increase of lung weight that occurred in tumor-removed mice (Figure 26). The number of lymphocytes in the spleens of LLC-removed mice was reduced to 5.30±1.03 x 10^6 from 2.11±0.41 x 10^7 cells in normal mice. The splenic CD4+ and CD8+ T cells and natural killer (NK) cells in tumor-removed mice were reduced compared to those of normal mice. 4-Hydroxyderricin (50 mg/kg twice daily) inhibited the reduction of lymphocyte, CD4+ and CD8+ T cells and NK cell number in tumor-removed mice. On the other hand, the numbers of lymphocytes, CD4+ and CD8+ T cells and NK cells were further reduced by intraperitoneally-administered doxorubicin compared to untreated tumor-removed mice (Table IV).

In mice with intrasplenically-implanted LLC cells, the weight of the tumor in the spleen was reduced by oral administration of 4-hydroxyderricin at a dose of 50 or 100 mg/kg for 19 days (Figure 27). Primary tumor cells in the spleen grew and invaded the white and red pulp of the spleen. Orally-administered 4-hydroxyderricin (50 or 100 mg/kg) prevented tumor growth and invasion of the white pulp of the spleen, and it induced the accumulation of lymphocytes around the tumor.

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**Figure 29.** Effects of ergosterol isolated from *Agaricus blazei* on tumor growth in sarcoma 180-bearing mice. Solid-type sarcoma 180 was prepared by subcutaneous transplantation into the right abdomen of mice on day 0. The indicated amounts of ergosterol were administered orally for 20 consecutive days. Values are means±S.E. of 10 mice.

**Figure 30.** Effects of sodium pyroglutamate isolated from *A. blazei* on tumor growth in LLC-bearing mice. Values are means±SE of 8 mice. *Significantly different from untreated LLC-bearing mice, p<0.05.

**Table V.** Effects of ergosterol on the weights and hemoglobin contents in the gels 5 day after implantation into mice of Matrigel supplemented with acidic fibroblast growth factor (aFGF) and heparin.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Matrigel weight (mg)</th>
<th>Hemoglobin content (mg/Matrigel)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrigel alone</td>
<td>103.16±10.15*</td>
<td>2.6±0.68*</td>
</tr>
<tr>
<td>Matrigel + aFGF (1 ng/mL) + heparin (64 U/mL)</td>
<td>371.60±39.75</td>
<td>21.0±4.00</td>
</tr>
<tr>
<td>Matrigel/aFGF/heparin</td>
<td>185.58±4.40*</td>
<td>6.4±1.86*</td>
</tr>
<tr>
<td>Matrigel/aFGF/heparin + ergosterol (400 µg/mL)</td>
<td>108.84±9.69*</td>
<td>3.8±0.58*</td>
</tr>
<tr>
<td>Matrigel/aFGF/heparin + ergosterol (800 µg/mL)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1*C57BL/6 J mice were each injected subcutaneously with 0.5 mL of Matrigel supplemented 1 ng/mL aFGF and 64 U/mL heparin in the absence or presence of ergosterol (400 and 800 µg/mL).

2Each value represents the means±S.E. of five mice.

*Significantly different from Matrigel/aFGF/ heparin-treated mice.
cells in the white pulp of the spleen (Figure 27). Mice with intrasplenically-implanted LLC had tumor metastasis to the liver. 4-Hydroxyderricin (50 or 100 mg/kg) tended to reduce the number of tumor cell colonies that metastasized to the liver compared with the number in mice bearing intrasplenic LLC tumors, although the effect was not significant. Metastatic tumor cells in the liver grew and invaded the region containing hepatocytes in mice with intrasplenically-implanted LLC. The growth of metastatic tumors in the livers of mice with intrasplenically-implanted LLC was also inhibited by orally-administered 4-hydroxyderricin (50 or 100 mg/kg). Lymphocytes also accumulated around the metastatic tumor cells in the liver of mice treated orally with 4-hydroxyderricin (Figure 28).

Figure 31. Anti-angiogenic effects of sodium pyroglutamate isolated from A. balzei. Effects of sodium pyroglutamate on Matrigel weight (a) and hemoglobin concentration (b) of gels induced by Matrigel supplemented with heparin and VEGF. Values are means±SE of 6 mice in each group. c) Photographs of Matrigel 6 days after subcutaneous injection of Matrigel alone or Matrigel supplemented with heparin and VEGF in the absence or presence of the indicated amounts of sodium pyroglutamate.
4-Hydroxyderricin had no effect on DNA synthesis in LLC cells and HUVECs at 0.1 to 100 μM (data not shown). 4-Hydroxyderricin inhibited the Matrigel-induced formation of capillary-like tube formation of HUVECs at 10-100 μM in vitro (data not shown). From these results, it seems likely that the mechanism of antitumor and antimetastatic actions of 4-hydroxyderricin might involve inhibition of tumor-induced angiogenesis.

Thus, the difference of side chain in the structure of xanthoangelol and 4-hydroxyderricin may contribute to the mechanisms of antitumor and antimetastatic actions. Further studies are needed to clarify this point.
Antitumor and antimetastatic actions of active substances isolated from Basidiomycete fungus

Basidiomycetes such as *Agaricus blazei*, *Ganoderma lucidum*, *Lentinula edodes* and *Polyporus umbellatus* have been used by about 1,000,000 to 2,000,000 people in Japan for the prevention of cancer and/or as an adjuvant with cancer chemotherapy drugs after the removal of malignant tumors. There have been a number of reports that many basidiomycetes have antitumor activity in sarcoma 180-bearing mice. It has been reported that the polysaccharide (β-glucan) fractions of various basidiomycetes have potent antitumor actions and that this antitumor mechanism of polysaccharide fractions might be due to the inhibition of tumor growth through the enhancement of immune function. The antitumor or antimetastatic actions of low molecular weight substances isolated from *A. blazei* and *G. lucidum* are reviewed.

The basidiomycete fungus *A. blazei* Murill (Japanese name: Himematsutake or Agarikustake) has been traditionally used as a health food source in Brazil for the prevention of cancer, diabetes, hyperlipidemia, arteriosclerosis and chronic hepatitis. It has been reported that 200,000 to 400,000 kg of the dried body of *A. blazei* is produced every year in Japan. *A. blazei* is used by 300,000 to 500,000 persons for the prevention of cancer and/or as an adjuvant with cancer chemotherapy drugs after the removal of a malignant tumor. The hot water extract of *A. blazei* has potent antitumor activity in sarcoma 180-bearing mice (29-32), and the antitumor substance was postulated to be the β-(1-6)-glucan fraction. However, the antitumor effects of low molecular weight fractions have not been well studied. Two low molecular weight substances, ergosterol and sodium pyroglutamate, were isolated from this basidiomycete fungus as antitumor and anti-angiogenic substances (33, 34). Oral administration of ergosterol (200, 400 and 800 mg/kg) for 20 days reduced tumor volume (Figure 29). The inhibition ratios for tumor growth with oral administration of ergosterol at the doses of 100, 200, 400 and 800 mg/kg for 20 days were 0.0±30.6, 62.3±8.8, 70.9±10.8 and 85.5±4.7%, respectively. Tumor volume was significantly reduced by oral administration of sodium pyroglutamate (30, 100 and 300 mg/kg) for 30 consecutive days compared to that of untreated LLC-bearing mice (Figure 30). To clarify the antitumor action of ergosterol and sodium pyroglutamate,
Figure 34. Effects of triterpenoid fraction isolated from *Ganoderma lucidum* on tumor weight in mice with intrasplenically-implanted LLC. Values are means ± S.E. of 5-8 mice in each group. The sham-operated group consisted of 5 mice; the LLC-bearing groups (control) and the triterpenoid fraction-treated group both consisted of 8 mice.

Figure 35. Effects of triterpenoid fraction isolated from *Ganoderma lucidum* on liver metastasis in mice with intrasplenically-implanted LLC. Values are means ± S.E. of 5-8 mice.

the inhibitory effect on angiogenesis induced by Matrigel supplemented with acidic FGF (aFGF) or VEGF was examined. Ergosterol (400 and 800 Ìg/mL) and sodium pyroglutamate (200, 400 and 800 Ìg/mL) inhibited increases in the weight and hemoglobin concentration of the gels (Table V and Figure 31).

In addition, the effects of sodium pyroglutamate on the immunohistochemistry of tumors in mice with subcutaneously-implanted LLC were examined. Sodium pyroglutamate (30, 100 and 300 mg/kg) increased the number of apoptotic cells in the tumors by day 31 in mice with subcutaneously-implanted LLC. In the untreated LLC-bearing mice, von Willebrand factor (vWF) expression was increased in the tumors. This finding shows that neovascularization was induced together with tumor growth. Angiogenesis in the tumors was inhibited by the oral administration of sodium pyroglutamate (30, 100 and 300 mg/kg). CD8+ T cells invaded the central area of the tumors after the oral administration of sodium pyroglutamate (30, 100 and 300 mg/kg). Natural killer (NK) cells also invaded the central area of the tumors after the oral administration of sodium pyroglutamate (30, 100 and 300 mg/kg) (Figure 32 and Table VI). Moreover, sodium pyroglutamate (30, 100 and 300 mg/kg) significantly inhibited the number of tumor colonies metastasizing to the lung compared to that in untreated mice with intravenous injection of LLC (Figure 33).

The fruit body of *Ganoderma lucidum* (Fr.) Karst has been used for the treatment of hypertension, hyperlipidemia, arthritis, bronchitis, arteriosclerosis, diabetes and cancer. In a previous paper, it was reported that an aqueous extract of *G. lucidum* reduced the elevation of blood glucose without elevating blood insulin in tests utilizing intravenous infusion of epinephrine and oral infusion of glucose (35). The polysaccharide fractions of *G. lucidum* have potent antitumor activities in tumor-bearing animals (36-40). Recently, Min et al. (41) reported that triterpenes isolated from the spores of *G. lucidum* have cytotoxicity against Meth-A and LLC in vitro. However, the antitumor and antimetastatic actions of the triterpenoid fraction of *G. lucidum* have not yet been studied in vivo in animal models. The effects of the triterpenoid fraction isolated from *G.
lucidum using mice implanted intrasplenically with LLC were examined (42). The isolated triterpenoid fraction (100 and 200 mg/kg) for 19 days inhibited tumor weight in the spleen (Figure 34). Moreover, the triterpenoid fraction (100 and 200 mg/kg) significantly reduced the number of tumor cell colonies that metastasized to the liver compared with the number in untreated LLC-bearing mice (Figure 35). The triterpenoid fraction inhibited the angiogenesis induced by Matrigel supplemented with VEGF. Therefore, the isolation of anti-angiogenic substance(s) from the triterpenoid fraction of G. lucidum was performed using the Matrigel/VEGF-induced angiogenesis model in situ. An anti-angiogenic substance was isolated from the triterpenoid fraction, identified as ganoderic acid F based on the data of IR, $^1$H and $^{13}$C-NMR and MS analyses (Figure 36). Ganoderic acid F (800 μg/mL), isolated from G. lucidum, inhibited the angiogenesis induced by Matrigel supplemented with heparin and VEGF (Figure 37).

Thus, it is suggested that the low molecular weight substances such as ergosterol, sodium pyroglutamate (isolated from Agaricus blazei) and ganoderic acid F (isolated from the triterpenoid fraction of Ganoderma lucidum) have antitumor and/or antimetastatic actions by the inhibition of tumor-induced angiogenesis in in situ and/or in vivo experiments.

References


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