Oral Picropodophyllin (PPP) Is Well Tolerated In Vivo and Inhibits IGF-1R Expression and Growth of Uveal Melanoma

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PURPOSE. The cyclolignan picropodophyllin (PPP) efficiently blocks the activity of insulinlike growth factor-1 receptor (IGF-1R) and inhibits the growth of uveal melanoma cells in vitro and in vivo. In this study, the authors investigated the efficiency of orally administered PPP on the growth of uveal melanoma xenografts. In addition, they focused on the effect of PPP on vascular endothelial growth factor (VEGF) in vivo and evaluated its effects in combination with other established antitumor agents in vitro.

METHODS. Four different uveal melanoma cell lines (OCM-1, OCM-3, OCM-8, 92-1) were treated with PPP alone and in combination with imatinib mesylate, cisplatin, 5-fluorouracil, and doxorubicin. Cell viability was determined by XTT assay. SCID mice that underwent xenografting with uveal melanoma cells were used to determine antitumor efficacy of oral PPP in vivo. Five mice were used per group. Tumor samples obtained from the in vivo experiments were analyzed for VEGF and IGF-1R expression by Western blotting.

RESULTS. PPP was found to be superior to the other antitumor agents in killing uveal melanoma cells in all four cell lines ($IC_{50} < 0.05\ \mu M$). Oral PPP inhibited uveal melanoma growth in vivo in OCM-3 ($P = 0.03$) and OCM-8 ($P = 0.01$) xenografts and was well tolerated by the animals. PPP decreased VEGF expression in the OCM-1 ($P = 0.006$) and OCM-8 ($P = 0.01$) tumors.

CONCLUSIONS. Oral PPP was well tolerated in vivo, caused total growth inhibition of uveal melanoma xenografts, and decreased VEGF levels in the tumors. (*Invest Ophthalmol Vis Sci.* 2008;49:2337–2342) DOI:10.1167/iovs.07-0819

Uveal melanoma is the most common primary intraocular malignant tumor in adults. It carries a high mortality rate because of the high incidence of metastases, which occur preferentially to the liver.[1,2] Current management usually consists of enucleation or radiation of the primary tumor. There are no postoperative adjuvant therapies available to decrease the risk for metastasis because none of the current treatments against metastatic uveal melanoma has yet shown to be effective.[3] Therefore, new molecular targets must be established to provide effective treatment modalities.

Previously, we detected the expression of IGF-1R in clinical samples of uveal melanoma and showed that the high expression level of IGF-1R correlated significantly with poor survival.[4] Moreover, we found that cultured uveal melanoma cells responded to the inhibition of insulinlike growth factor-1 receptor (IGF-1R)—such as by using neutralizing antibodies—with cell death.[5] These data pointed to the possibility of using IGF-1R as a target for uveal melanoma. Recently, we demonstrated that picropodophyllin (PPP), an inhibitor of the IGF-1R tyrosine phosphorylation in vitro and in vivo,[6–11] efficiently decreased the survival of cultured uveal melanoma cells and blocked the establishment and growth of uveal melanoma tumor xenografts.[12] Furthermore, PPP drastically decreased the development of hepatic metastasis.[12] PPP was proved to inhibit the phosphorylation of IGF-1R and downstream molecules (ERK and Akt) in the uveal melanoma tissues.[12] In addition, PPP-treated malignant cells cannot acquire resistance to PPP or cross-resistance to conventional cytostatic drugs.[13]

PPP has recently been demonstrated to attenuate IGF-1R–dependent VEGF secretion in vitro.[14,15] It blocks VEGF at the level of transcription, suggesting that inhibits the synthesis of VEGF.[14] Because VEGF induces neovascularization, this effect of PPP may contribute to the inhibition of tumor growth.

In all in vivo studies performed thus far on solid tumors, PPP has been administered by intraperitoneal (IP) injection.[7,12] However, for future clinical use, it is desirable to administer PPP orally. This strategy has several benefits. One is that it allows treatment in outpatient settings. In fact, one study using PPP orally has recently been performed on the mouse multiple myeloma model 5T2MM.[16] It was found that oral PPP prolonged the survival of mice with established disease to almost 3 months compared with untreated controls.[16] The mice were treated daily with oral PPP for up to 150 days, implying that the drug is well tolerated in vivo. In contrast, longer treatments with intraperitoneal PPP led to peritonitis and peritoneal fibrosis, which impairs absorption of the drug (data not shown).

In this study, we investigated the effect of PPP in vitro in combination with some conventional antitumor agents in vitro on uveal melanoma cell lines. We also aimed to investigate the effects of oral administration of PPP on uveal melanoma xenografts. In addition, we were interested in studying the effects of PPP on VEGF expression in the tumors.

MATERIALS AND METHODS

Reagents

PPP was synthesized as described[7] and was dissolved in dimethyl sulfoxide (0.5 mM) before it was added to cell cultures. The phospho-tyrosine (PY99) and polyclonal antibodies to the β-subunit of IGF-1R (H-60) were from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). Imatinib mesylate (Gleevec) was a gift from Novartis Pharmaceuticals (Basel, Switzerland).

Cell Cultures

Four cell lines obtained from human primary uveal melanomas (OCM-1, OCM-3, OCM-8, 92–1) were previously described.[5] R-vsrc were a gift from Renato Baserga (Thomas Jefferson University, Philadelphia, PA). The R-vsrc fibroblasts are R cells transfected with the v-src and have insulin receptor (IR) substrate-1 and Shc constitutively tyrosine phosphorylated.[17]

Cell Survival Assay

Cell viability determinations were performed using a cell proliferation kit (Cell Proliferation Kit II; Roche Inc., Indianapolis, IN), which is based on colorimetric change of the yellow tetrazolium salt XTT in orange formazan dye by the respiratory chain of viable cells.[18]

Immunoprecipitation and Western Blotting

Tumor samples obtained from the in vivo experiments with OCM-1 and OCM-8 cells were analyzed. Samples from fresh-frozen tumors from drug- and solvent-treated mice were cut in pieces and suspended in freshly prepared homogenization buffer, as described.[7] After centrifugation at $14,000g$ for 10 minutes at 4°C, the supernatants were immunoprecipitated for IGF-1R and IR, and determination of IGF-1R and IR phosphorylation, after indicated treatments, was completed. Fifteen microliters of protein G plus-A/G agarose and 1 μg antibody were added to 1 mg protein material. After overnight incubation at 4°C on a rocker platform, the immunoprecipitates were collected by centrifugation in a microcentrifuge at 2500 rpm for 2 minutes, as described.[19] The supernatant was discarded, whereupon the pellet was washed and then dissolved in a sample buffer for SDS-PAGE.

Twenty micrograms of protein per sample were electrophoresed in a 10% Tris–glycine gel (Novex; Invitrogen, Carlsbad, CA). After electrophoresis, the proteins were transferred overnight to nitrocellulose membranes (Amersham, Uppsala, Sweden) and then were blocked for 1 hour at room temperature in a solution of 5% (wt/vol) skimmed milk powder and 0.02% (wt/vol) Tween 20 in PBS, pH 7.5. Incubation with appropriate primary antibodies for IGF-1R, IR, and VEGF was performed for 1 hour at room temperature or overnight at 4°C. This was followed by washes with PBS and incubation with either an HRP-labeled or a biotinylated secondary antibody (Amersham, Amersham, UK) for 1 hour. After the biotinylated secondary antibody, incubation with streptavidin-labeled horse peroxidase was performed. Detection was made with either enhanced chemiluminescence, Amersham, or Pierce (SuperSignal West Pico; Pierce, Rockford, IL) reagents. The films were scanned (Fluor-S; Bio-Rad, Hercules, CA).

In Vivo Experiments

Ten-week-old pathogen-free SCID mice were used and housed within plastic isolators in a sterile facility. R-vsrc, OCM-3, and OCM-8 cells were injected subcutaneously at 10^7 cells/mice in a 0.2-mL volume of sterile saline solution. At indicated time points the mice were given PPP, which was mixed in food powder (3.2 mg PPP/5 g food/mouse), whereas the control mice received control vehicle mixed in the food. The animals ate 5 g food per PPP mixture or 5g food per day. The concentration of the administered PPP was monitored in the serum up to 8 hours after the food was given, and serum concentrations stayed above 10 μM (data not shown). Experimental treatments with PPP were performed, as previously described.[7] All animal experiments

TABLE 1. Effect of PPP and Other Agents on Cell Survival

Cell Line	IC_{50}			
	OCM-1	OCM-3	OC8	92-1
PPP (μM)	<0.05	<0.05	<0.05	<0.05
Imatinib mesylate (μM)	0.2	0.2	0.3	0.2
Cisplatin (μM)	5.5	6.2	1.8	2.2
5-FU (mM)	>5	5	>5	>5
Doxorubicin (μM)	>10	>10	>10	>10

The four uveal melanoma cell lines OCM-1, OCM-3, OCM-8, and 92-1 in triplicate were treated with different concentrations of PPP, imatinib mesylate, cisplatin, 5-FU, or doxorubicin for 48 hours and analyzed for cell survival with XTT assay. Based on the received dose-response results, the IC_{50} values for each cell line/drug were determined. The experiments were repeated several times with similar results.

followed the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Animal Care Committee of Karolinska Institute.

Statistical Analysis

All results are expressed as mean ± SD. Results were compared using analysis of variance (ANOVA) and Student's t-test. A confidence level of $P < 0.05$ was considered statistically significant.

RESULTS

Effects of PPP on Cell Survival Compared with Other Antitumor Agents

The four uveal melanoma cell lines OCM-1, OCM-3, OCM-8, and 92–1 were analyzed for cell survival after treatment with PPP, in comparison with imatinib mesylate (a Kit kinase inhibitor) and the three cytostatic agents cisplatin, 5-fluorouracil (5-FU), and doxorubicin. Results are presented in Table 1. As shown, all four cell lines responded strongly to PPP, and in all experiments (total, 12), the IC_{50} values were lower than 0.05 μM. The cell lines also responded to imatinib mesylate, as previously reported,[20] with IC_{50} values of 0.2 to 0.3 μM. Cisplatin decreased cell survival most efficiently in OCM-8 and 92–1 cells (IC_{50}, 1.3 and 2.2 μM, respectively). All cell lines incubated with 5-FU, with the exception of OCM-3, exhibited IC_{50} exceeding 5 mM. Doxorubicin was least efficient, and doses of 10 μM were not sufficient to kill 50% of the cells in any cell line (Table 1). We could not find any general additive or synergistic effects when combining PPP with any of the other drugs. However, there were some exceptions. A concentration of PPP causing less than 25% cell death lowered the IC_{50} of doxorubicin to 2 μM in OCM-3 cells, and it increased the sensitivity to cisplatin and 5-FU in OCM-8 (from 1.8 to 0.3 μM and from >5 to 0.4 mM, respectively).

Effect of Oral PPP on Xenografts

PPP was mixed in food at a concentration of 32 mg/50 g food. A SCID mouse was estimated to consume approximately 5 g food/day. SCID mice were inoculated with uveal melanoma cells subcutaneously. The tumors was established when they reached sizes of 100 to 200 mm^3 and grew steadily. One group of mice received control food, and the other group received food with PPP. In the first experiment mice, with established OCM-3 xenografts were treated with PPP for 16 days. As shown in Figure 1, the tumors responded to PPP with drastic growth inhibition. At the end of the experiment, the tumors remained at the same sizes as at the beginning of the experiment ($P =$
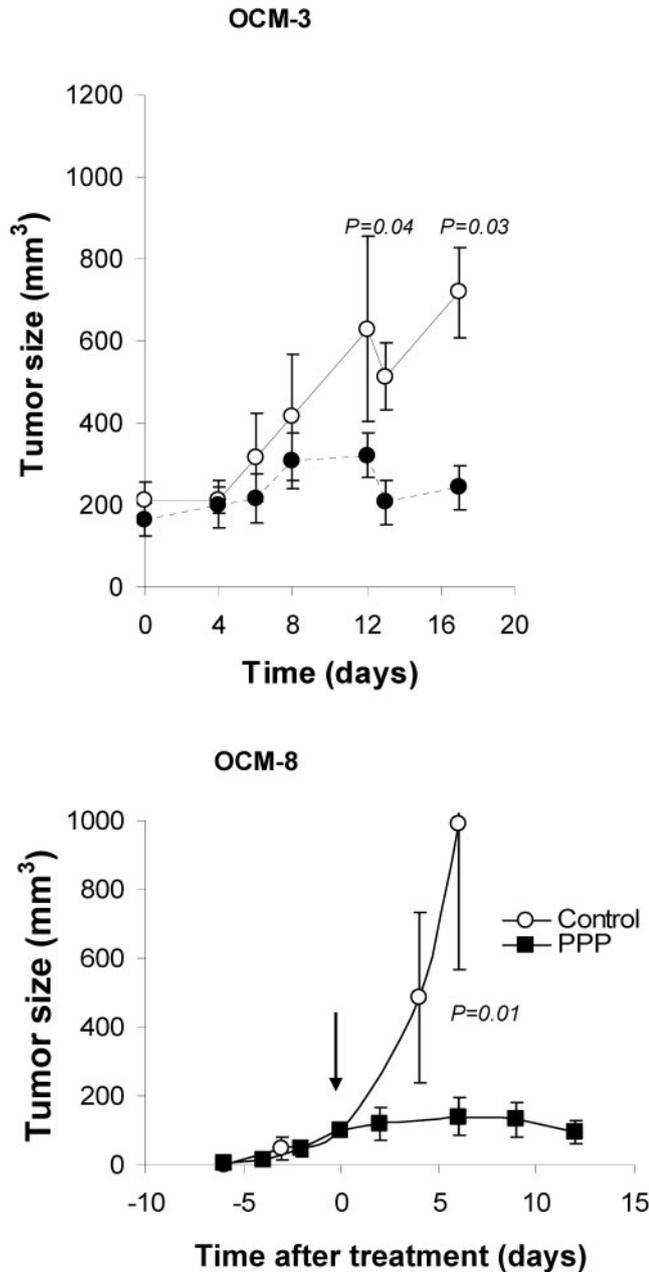
OCM-3

OCM-8

FIGURE 1. Effects of oral PPP on uveal melanoma xenografts in mice. SCID mice were subcutaneously inoculated with OCM-3 (**A**) and OCM-8 (**B**) cells. Each group in both experiments was composed of five mice. When the tumors had become established, the mice were fed daily with control food or food containing PPP for 17 days (OCM-3) or up to 12 days (OCM-8).

0.03). Similar results were obtained in an experiment on mice with established OCM-8 tumors ($P = 0.01$; Fig. 1B). Experiments were also performed on mice with xenografts established from OCM-1 cells. The results were similar to those of OCM-3 and OCM-8 tumors (data not shown).

Previously, we demonstrated that PPP did not affect the tumor growth of established xenografts composed of IGF-1R–negative compared with rc-transformed murine fibroblast (R-vsrc), whereas IGF-1R–positive xenografts were fully responsive.[7] These results suggested that PPP is selective for IGF-1R regarding tumor growth in vivo. Here we also investigated the effect of PPP on SC R-vsrc xenografts, with the difference that the mice were exposed daily to food containing PPP. The first

experiment was made on established tumors (size range, 100 – 250 mm³). One group of mice received control food and the other received food containing PPP. The end point of this study was set to the time when the first group exhibited a tumor size of 1000 mm³. Consistent with our previous study,[7] tumors in both groups reached this size simultaneously (Fig. 2). Thus, established IGF-1R–negative tumors were not affected by PPP. We also conducted an experiment on early nonestablished R-vsrc tumors to investigate whether PPP may affect the establishment of tumor. R-vsrc cells were inoculated, and, as
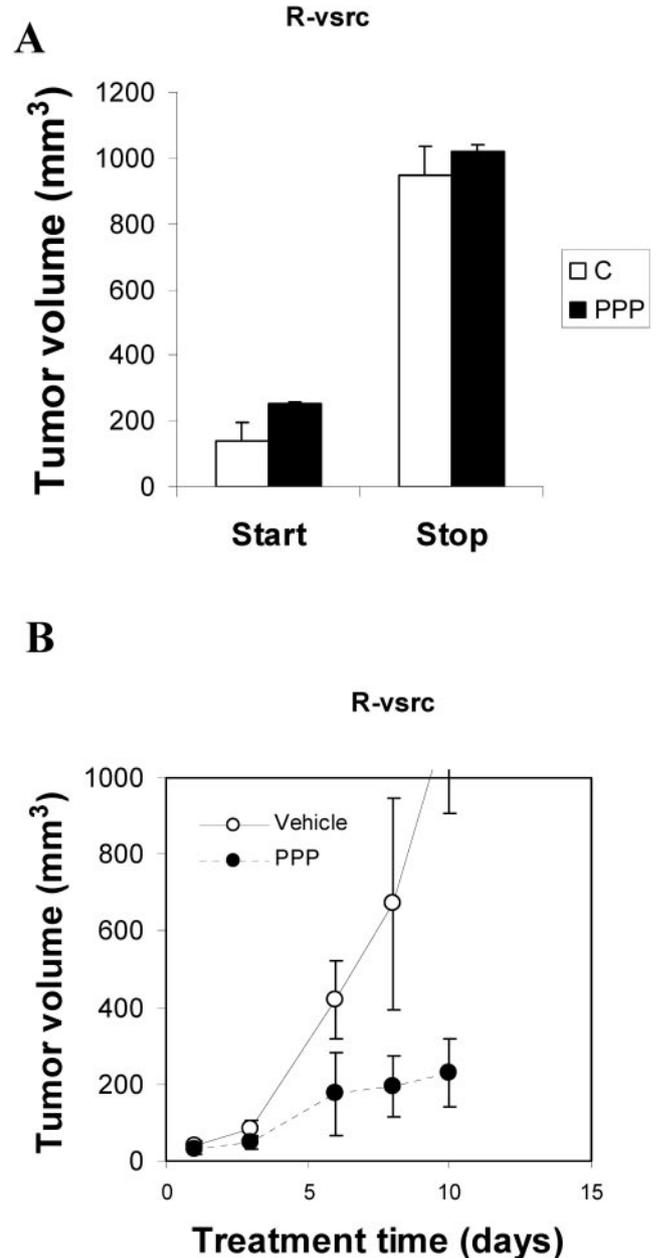
R-vsrc

A

B

R-vsrc

FIGURE 2. Effect of PPP on IGF-1R–negative cells. (**A**) SCID mice were inoculated subcutaneously with R-vsrc cells. Five mice were included in each group. When the tumors had become established, the mice were fed daily with either control food or food containing PPP. The experiment was stopped simultaneously for both groups when the first group reached an average size of 1000 mm³, and the tumor sizes were compared. (**B**) SCID mice were inoculated with R-vsrc cells subcutaneously Five mice were included in each group. Immediately after the tumors became palpable (1–2 mm³), the mice were fed for 10 days with control food or food containing PPP.
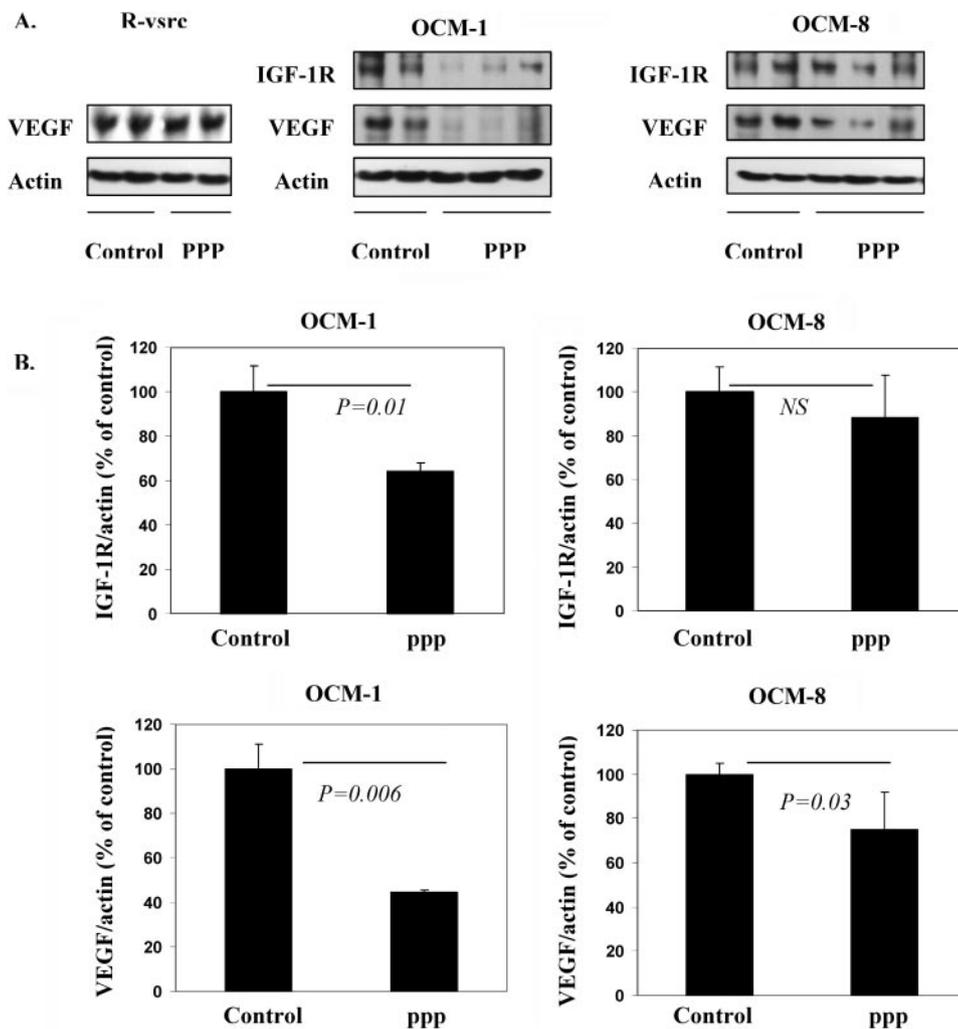
FIGURE 3. Effect of PPP on VEGF and IGF-1R expression. (**A**) SCID mice were inoculated subcutaneously with R-vsrc, OCM-3, and OCM-1 cells. Five mice were included in each group. When the tumors had become established, the mice were fed daily with either control food or food containing PPP for 14 days (R-vsrc and OCM-3) and 21 days (OCM-1). Mice were killed and tumors snap frozen were later analyzed for the expression of IGF-1R and VEGF. Actin was used as a loading control. (**B**) Quantification of IGF-1R and VEGF expression, normalized to the β-actin expression, of the experiments shown in (**A**). Mean ± SD and results of statistical analysis are shown.

soon as small tumors could be palpated (1–2 mm³ in size), the mice were treated as described. The experiment stopped when tumors of any group had reached a size of 1000 mm³. At that time, all mice were killed. Figure 2B shows that tumors of both groups of mice established properly and increased in size until the end of the experiment. However, the tumors of the control group grew much faster. At day 10, the control tumors had reached a mean size of 1000 mm³, whereas the size of the PPP group was 200 mm³. This difference might be explained by the fact that PPP has different effects apart from inhibiting IGF-1R, and these effects emerge clearly in this experimental model (tumor establishment).

Effect on VEGF Expression In Vivo

The effects of PPP on the expression of VEGF and IGF-1R in tumor samples were analyzed and compared with the expression of β-actin. Tumor tissues (R-vsrc, OCM-8, and OCM-1) from mice exposed to control food and PPP-containing food were snap frozen. Protein lysates were prepared and analyzed for expression of VEGF and IGF-1R (not for R-vsrc tumors). VEGF expression, normalized to the β-actin expression, in the PPP groups for both OCM-1 and OCM-8 xenografts was strongly and moderately, respectively, decreased compared with the controls (Fig. 3). On the other hand, PPP did not affect VEGF expression in R-vsrc tumors, supporting specificity for the IGF-1R in this context (Fig. 3). There was also a decreased expression of IGF-1R, especially in the OCM-1 tumors, whereas

in the case of OCM-8 tumors, the IGF-1R was not significantly decreased (Fig. 3). This result is consistent with a recent study showing that PPP not only inhibits the activity of IGF-1R, it also causes the downregulation of the receptor.[21]

DISCUSSION

Uveal melanoma cells seem to be more sensitive to PPP than many other tumor cells. The IC_{50} values in all four cell lines studied were lower than 0.05 μM. By combining PPP with imatinib mesylate or a cytostatic agent such as cisplatin, 5-FU, or doxorubicin, we could only detect a weak synergistic effect with cisplatin and 5-FU in OCM-8 cells and with doxorubicin in OCM-3. These data are consistent with other observations that the inhibition of IGF-1R may sensitize malignant tumors to cytostatic agents.[22] However, it is obvious that PPP by itself is superior to the other agents in uveal melanoma cells. For that reason, we only focused on PPP as a monotherapeutic strategy in the following in vivo experiments. Actually, we also tested imatinib mesylate on uveal melanoma xenografts because it showed good effects in vitro but it did not cause any significant antitumor effects (data not shown).

PPP has proven to drastically reduce solid tumor size in vivo when administered intraperitoneally.[7,12] A recent study showed that oral PPP prolongs survival considerably in a model of multiple myeloma.[16] In the present study, we evaluated the effect of orally administered PPP on uveal melanoma xeno-

grafts. PPP efficiently blocked the growth of melanoma tumors. None of the PPP-treated mice exhibited any signs of toxicity or weight loss, and they maintained good appetites throughout the experiments. From a clinical perspective, switching from systemic to oral administration has several advantages. Because PPP has a short half-life (2–4 hours) in vivo (data not shown), it is probable that the treatment of humans would require permanent infusion during the entire treatment period. Such procedures can only be performed on supervised inpatients. In addition, daily intravenous injections will require professional (qualified) supervision. Thus, oral administration will lower the costs for public health care by reducing the required days of hospitalization. In addition, such administration is beneficial for the quality of life of the cancer patients.

For anticancer treatment, it is vital that an IGF-1R inhibitor not cross-react with the IR. Coinhibition of the IR would eventually lead to diabetogenic responses in vivo, a very serious side effect that obviously cannot be overcome by insulin treatment. Previous results have clearly shown that PPP does not inhibit the IR in vitro or in vivo and that it does not increase the levels of serum glucose under conditions in which the drug causes antitumor effects.[7,15]

Compared with our previous study showing that PPP causes full tumor regression of uveal melanoma xenografts,[12] the oral administration strategy resulted only in the inhibition of complete tumor growth, though there was a trend for some regression of the OCM-8 tumors (Fig. 2B). This difference is probably attributed to the fact that the current technique to mix PPP into the food is not the most optimal one, even if it works excellently in the multiple myeloma mouse 5T2MM model.[16] Actually, PPP prolonged the survival of the mice with myeloma to 3 months.[16] However, the myeloma study was performed on immunocompetent mice, whereas the present study was conducted on immunodeficient SCID mice.

One aspect that could contribute to a higher tumor sensitivity in the immunocompetent animals is that the host's immune response appears to be involved in the antitumor effect caused by IGF-1R inhibition.[23–25] Another explanation is that SCID mice may experience lower intestinal absorption of the drug, or it may be simply that the eating habits of the mouse types differ. The present results are favorable and give support for the oral use of PPP in the treatment of cancer. An optimal oral formulation (e.g., capsules) for clinical use remains to be developed.

We also studied the specificity for PPP regarding tumor growth. We found that, in line with previous results,[7] PPP did not affect the growth of established R-vsrc tumors in mice. Given that R-vsrc is IGF-1R deficient, this supports the idea that PPP blocks tumor growth mainly by inhibiting the IGF-1R. Permanent treatment with PPP also allowed the development of tumors (tumorigenesis). This result should be compared with our recent finding that only 3-day treatment drastically delayed (9 days) tumorigenesis of inoculated uveal melanoma cells.[12] In contrast, tumorigenesis in the PPP-treated mice started at the same time as in the controls (Fig. 3B). However, the rate of tumor growth differed in the sense that the xenografts of treated mice grew significantly more slowly. These results could have several explanations. PPP could have other effects than inhibiting the IGF-1R, and such unspecific effects could have emerged clearly in this experimental model (tumor development). However, it should be remembered that tumorigenesis is dependent on favorable host-tumor cell interactions.[26] Because the host (mouse) is not IGF-1R deficient, PPP could impair such functions that are important for optimal host-tumor cell interactions through its inhibitory effect on the IGF-1R. Previously, we found that PPP impairs cell adhesion and decreases the expression of several integrin molecules.[12] These results are inconsistent with those reported by others

using other strategies to inhibit the IGF-1R.[27] PPP could also inhibit IGF-1R–dependent expression of VEGF in the mice and in this way impair tumorigenesis.

IGF-1R–dependent expression of VEGF has previously been demonstrated to be blocked by PPP[14–16] and other IGF-1R inhibitors.[28] In this study, we also showed that PPP significantly inhibited VEGF expression in uveal melanoma xenografts, especially the OCM-1 xenografts, whereas the decrease in VEGF expression in OCM-8 xenografts was not as strong but was still significant. Actually, this is the first paper showing this response in solid tumors in vivo. In addition, PPP decreased the expression of IGF-1R in OCM-1 and OCM-8 xenografts, though this reduction was statistically significant only in the case of the OCM-1 xenografts. This could be caused by OCM-8 expressing less IGF-1R than OCM-1. In any case, the effect of PPP on tumor growth is not achieved only through direct IGF-1R inhibition but also through other mechanisms such as VEGF inhibition, as we show here, and MMP-2 inhibition.[12] The antiangiogenic effect mediated by the lowered VEGF levels could be an important component for the antitumor effect of PPP. In established R-vsrc, on the contrary, VEGF levels were not affected by PPP, supporting the notion that this response is IGF-1R specific.

In conclusion we showed that orally administered PPP is capable of blocking tumor growth in uveal melanoma xenografts. An important component for antitumor efficacy could be due to the inhibition of IGF-1R–dependent expression of VEGF. Because uveal melanoma seems to be especially sensitive to IGF-1R inhibition, our study points to the use of PPP in a monotherapeutic regimen in the treatment of this disease. However, clinical trials are required to confirm our hypothesis and results.

References

1. Rajpal S, Moore R, Karakousis CP. Survival in metastatic ocular melanoma. *Cancer.* 1983;52:334–336.
2. Diener-West M, Hawkins BS, Markowitz JA, Schachat AP. A review of mortality from choroidal melanoma, II: a meta-analysis of 5-year mortality rates following enucleation, 1966 through 1988. *Arch Ophthalmol.* 1992;110:245–250.
3. Damato B. Treatment of primary intraocular melanoma. *Expert Rev Anticancer Ther.* 2006;6:493–506.
4. Economou MA, All-Ericsson C, Bykov V, et al. Receptors for the liver synthesized growth factors IGF-1 and HGF/SF in uveal melanoma: intercorrelation and prognostic implications. *Invest Ophthalmol Vis Sci.* 2005;46:4372–4375.
5. All-Ericsson C, Girnita L, Seregard S, Bartolazzi A, Jager MJ, Larsson O. Insulin-like growth factor-1 receptor in uveal melanoma: a predictor for metastatic disease and a potential therapeutic target. *Invest Ophthalmol Vis Sci.* 2002;43:1–8.
6. Ulfarsson E, Karstrom A, Yin S, et al. Expression and growth dependency of the insulin-like growth factor I receptor in craniopharyngioma cells: a novel therapeutic approach. *Clin Cancer Res.* 2005;11:4674–4680.
7. Girnita A, Girnita L, del Prete F, Bartolazzi A, Larsson O, Axelson M. Cyclolignans as inhibitors of the insulin-like growth factor-1 receptor and malignant cell growth. *Cancer Res.* 2004;64:236–242.
8. Vasilcanu D, Girnita A, Girnita L, Vasilcanu R, Axelson M, Larsson O. The cyclolignan PPP induces activation loop-specific inhibition of tyrosine phosphorylation of the insulin-like growth factor-1 receptor: link to the phosphatidyl inositol-3 kinase/Akt apoptotic pathway. *Oncogene.* 2004;23:7854–7862.
9. Stromberg T, Ekman S, Girnita L, et al. IGF-1 receptor tyrosine kinase inhibition by the cyclolignan PPP induces G2/M-phase accumulation and apoptosis in multiple myeloma cells. *Blood.* 2006; 107:669–678.

10. Larsson O, Girnita A, Girnita L. Role of insulin-like growth factor 1 receptor signalling in cancer. *Br J Cancer.* 2005;92:2097–2101.

11. Rosengren L, Vasilcanu D, Vasilcanu R, et al. IGF-1R tyrosine kinase expression and dependency in clones of IGF-1R knock-out cells (R–). *Biochem Biophys Res Commun.* 2006;347:1059–1066.

12. Girnita A, All-Ericsson C, Economou MA, et al. The insulin-like growth factor-I receptor inhibitor picropodophyllin causes tumor regression and attenuates mechanisms involved in invasion of uveal melanoma cells. *Clin Cancer Res.* 2006;12:1383–1391.

13. Vasilcanu D, Weng WH, Girnita A, et al. The insulin-like growth factor-1 receptor inhibitor PPP produces only very limited resistance in tumor cells exposed to long-term selection. *Oncogene.* 2006;25:3186–3195.

14. Catrina SB, Botusan IR, Rantanen A, et al. Hypoxia-inducible factor-1alpha and hypoxia-inducible factor-2α are expressed in Kaposi sarcoma and modulated by insulin-like growth factor-I. *Clin Cancer Res.* 2006;12:4506–4514.

15. Menu E, Jernberg-Wiklund H, Stromberg T, et al. Inhibiting the IGF-1 receptor tyrosine kinase with the cyclolignan PPP: an in vitro and in vivo study in the 5T33MM mouse model. *Blood.* 2006;107:655–660.

16. Menu E, Jernberg-Wiklund H, De Raeve H, et al. Targeting the IGF-1R using picropodophyllin in the therapeutical 5T2MM mouse model of multiple myeloma: beneficial effects on tumor growth, angiogenesis, bone disease and survival. *Int J Cancer.* 2007;121:1857–1861.

17. Valentinis B, Morrione A, Taylor SJ, Baserga R. Insulin-like growth factor I receptor signaling in transformation by src oncogenes. *Mol Cell Biol.* 1997;17:3744–3754.

18. Girnita L, Girnita A, Larsson O. Mdm2-dependent ubiquitination and degradation of the insulin-like growth factor 1 receptor. *Proc Natl Acad Sci U S A.* 2003;100:8247–8252.

19. Girnita L, Shenoy SK, Sehat B, et al. Beta-arrestin and Mdm2 mediate IGF-1 receptor-stimulated ERK activation and cell cycle progression. *J Biol Chem.* 2007;282:11329–11338.

20. All-Ericsson C, Girnita L, Muller-Brunotte A, et al. c-Kit-dependent growth of uveal melanoma cells: a potential therapeutic target? *Invest Ophthalmol Vis Sci.* 2004;45:2075–2082.

21. Vasilcanu R, Vasilcanu D, Rosengren L, et al. Picropodophyllin induces downregulation of the insulin-like growth factor 1 receptor: potential mechanistic involvement of the Mdm2 E3 and β-arrestin1. *Oncogene.* 2008 Mar 6;27(11):1629–1638. Epub 2007 Sep 10.

22. Wan X, Harkavy B, Shen N, Grohar P, Helman LJ. Rapamycin induces feedback activation of Akt signaling through an IGF-1R-dependent mechanism. *Oncogene.* 2007;26:1932–1940.

23. Resnicoff M, Li W, Basak S, Herlyn D, Baserga R, Rubin R. Inhibition of rat C6 glioblastoma tumor growth by expression of *insulin*-like growth factor I receptor antisense mRNA. *Cancer Immunol Immunother.* 1996;42:64–68.

24. Resnicoff M, Sell C, Rubini M, et al. Rat glioblastoma cells expressing an antisense RNA to the insulin-like growth factor-1 (IGF-1) receptor are nontumorigenic and induce regression of wild-type tumors. *Cancer Res.* 1994;54:2218–2222.

25. Liu X, Turbyville T, Fritz A, Whitesell L. Inhibition of insulin-like growth factor I receptor expression in neuroblastoma cells induces the regression of established tumors in mice. *Cancer Res.* 1998;58:5432–5438.

26. Gallagher PG, Bao Y, Prorock A, et al. Gene expression profiling reveals cross-talk between melanoma and fibroblasts: implications for host-tumor interactions in metastasis. *Cancer Res.* 2005;65:4134–4146.

27. Felding-Habermann B. Integrin adhesion receptors in tumor metastasis. *Clin Exp Metastasis.* 2003;20:203–213.

28. Strammiello R, Benini S, Manara MC, et al. Impact of IGF-I/IGF-IR circuit on the angiogenetic properties of Ewing's sarcoma cells. *Horm Metab Res.* 2003;35:675–684.

E R R A T U M

Erratum in: Cover legend (*Invest Ophthalmol Vis Sci.* May 2008, Volume 49, Number 5).

The cover legend should read, "Area in the temporal retina of a 22-week gestation fetal human retina that was incubated with anti-CD39 (red). CD39 (ecto-ADPase) labels angioblasts and endothelial cells in the developing retinal vasculature. Angioblasts and endothelial cells in vascular cords also express CXCR4 (not shown in this image), which can be seen double labeled (red/green) in this same specimen in Figure 11 in Hasegawa et al. on page 2178 of this issue."