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# The effect of thalidomide on experimental tumors and metastases

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**Thalidomide has recently been shown to antagonize basic fibroblast growth factor-induced angiogenesis in the rat corneal micropocket assay. We have investigated the effect of thalidomide on growth, radiosensitivity and metastasis in murine SCCVII and Lewis Lung tumors. We found that daily thalidomide administration (0.77 mmol/kg/day, i.p.) does not alter primary tumor growth of SCCVII or Lewis Lung tumors. However, thalidomide administration does reduce radiosensitivity of the Lewis Lung tumor, and increases its sensitivity to combined treatment with radiation and the bioreductive cytotoxin tirapazamine. These findings suggest that thalidomide is elevating tumor hypoxia in the Lewis Lung tumor, presumably via an anti-angiogenic mechanism. We also found that thalidomde administration reduces the incidence of lung metastases from primary Lewis Lung tumors. Thalidomide may therefore have utility in the management of solid tumors, especially when combined with drugs that are selectively toxic to cells at reduced oxygen tension (e.g. bioreductive cytotoxins).**

*Key words:* Anti-angiogenesis, Lewis Lung, metastasis, neovascularization, thalidomide, radiation, SCCVII, tumor oxygenation.

## Introduction

Thalidomide (α-phthalimidoglutarimide) was used in the late 1950s and early 1960s as a sedative, and was withdrawn in 1961 when it was found to cause fetal abnormalities, particularly stunted limb development, when used by pregnant women.[1,2] Although the mechanism underlying the embryotoxicity of thalidomide remains unclear, it has recently been postulated to be related to inhibition of fetal neovascularization such as that occurring in the developing limb buds.[3] This postulate was developed after thalidomide was shown to be a potent inhibitor of basic fibroblast growth factor (bFGF)-induced angiogenesis when tested using the rat corneal micropocket assay.[3] Angiogenesis is a prerequisite for tumor growth[4] and, as such, has been identified as a potentially useful target for anticancer drug development.[5,6,7] The antiangiogenic activity of thalidomide prompted us to evaluate its effect on the growth, and the development of hypoxia, in transplanted tumors. If thalidomide interferes with the process of angiogenesis then tumor growth and tumor hypoxia may be modulated. Increased hypoxia within tumors potentiates the effectiveness of bioreductive cytotoxins, a new class of anticancer agents that have preferential toxicity to cells at reduced oxygen tension. Combined with radiation therapy, anti-angiogenic and bioreductive cytotoxins may constitute a therapeutic modality with improved efficacy against solid tumors. Bioreductive cytotoxins such as tirapazamine[8] and RB 6145[9] are undergoing clinical trials, and their antitumor activity can be enhanced by reducing tumor oxygenation.[10]

Clinical experience with thalidomide indicates that it does not significantly inhibit angiogenesis in humans. When used as a sedative 35 years ago, there were no reports of interference with wound healing or the cyclic neovascularization associated with the female reproductive organs. Although its effect on the developing fetus may have been angiogenesis related, it certainly did not halt vascular development. In 1965, Sheskin[11] observed that thalidomide improved erythema nodosum leprosum associated with lepromatous leprosy. As a consequence of this fortuitous discovery, thalidomide was believed to have immunosuppresive properties and, in addition to its use in reactional lepromatous leprosy,[12] clinical studies have, or are being conducted, in chronic discoid lupus erythematosus, Bechet's syndrome, prurigo nodularis, ulcerative colitis,[13] rheumatoid arthritis,[14] AIDS,[15] Kaposi's sarcoma[16] and graft-versus-host disease.[17] These conditions may involve neovascularization as part of the disease process and it is therefore possible

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that thalidomide is exerting an anti-angiogenic influence that contributes to its therapeutic efficacy.

## Materials and methods

### Chemicals

Thalidomide was purchased from Andrulis Pharmaceuticals (Beltsville, MD) and was administered i.p. as a 10 mg/ml suspension in 1% methyl cellulose. Vehicle-treated control animals received methyl cellulose solution. Tirapazamine (3-amino-1,2,4-benzotriazine-1,4 dioxide) was provided by Sanofi-Winthrop (Renssellaer, NY) and was administered by i.p. injection of a 7.5 mmol/l solution in saline. Media, serum and trypsin were obtained from Gibco/BRL (Burlington, Ontario, Canada). Methyl cellulose (M-0512), collagenase and DNase were obtained from Sigma (Mississauga, Ontario, Canada).

### Tumors and animals

The anaplastic squamous cell carcinoma (SCCVII) and Lewis Lung (LL) tumor were implanted s.c. ($10^6$ cells in a volume of 0.05 ml) into the sacral region of female C3H/hen and C57/b16 mice, respectively. Mice were bred and housed in our institutional animal facility, and used after local ethical committee approval. They were allowed free access to food and water. Irradiation was performed as previously described.[10]

### Tumor control assays

Growth was monitored by measuring three orthogonal tumor dimensions (*a*, *b* and *c*) three times per week with vernier calipers. Tumor volume was calculated as $\pi/6(a \cdot b \cdot c)$. The cell survival assay was performed as previously described[10] except that MEM with Earles salts containing 10% fetal bovine serum was used to grow SCCVII cells and RPMI containing 10% fetal bovine serum was used to grow LL cells. Single cell suspensions were prepared by incubating minced tumor tissue with 2 mg/ml trypsin, 0.32 mg/ml collagenase and 0.24 mg/ml DNase in 5 ml phosphate-buffered saline.

## Results

Figure 1 shows that thalidomide has no influence on the growth of primay LL and SCCVII tumors when administered i.p. at a dose of 0.77 mmol/kg/day starting the day of tumor implantation. When LL tumors were irradiated *in situ* the tumors of animals that had received thalidomide daily from the day of implant appeared to be radioresistant compared with the tumors on the control, vehicle administered animals. After local irradiation with 20 Gy X-rays, the tumors of the vehicle-treated mice darkened and thick scabs formed which persisted for a minimum of 10 days. The tumors of the thalidomide-treated animals darkened after irradiation but did not form scabs and continued to grow after a delay of 2–4 days. When the lungs of these mice were examined 10 days after irradiation, we found that the thalidomide-treated mice had significantly fewer metastases than the control, vehicle-treated animals (Figure 2). The radioresistance exhibited by the tumors of thalidomide-treated animals probably reflected the presence of an increased proportion of hypoxic cells within the tumor. To test this we performed an experiment using both SCCVII and LL tumors where the tumors were locally irradiated *in situ* and cell survival was assessed *in vitro*. Once again, mice were administered thalidomide daily (0.77 mmol/kg/day) from the day of implantation of the tumors and the tumors were irradiated with 25 Gy X-rays after 15 days at which time the mean diameter (±SD) of the tumors was $151 \pm 75$ mm$^3$.

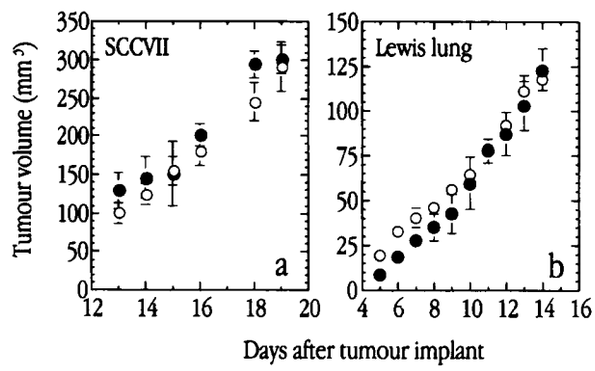


**Figure 1.** Effect of thalidomide on the growth of SCCVII tumors (a) and (LL) tumors (b). Closed symbols represent the tumor volumes from mice administered thalidomide suspension (0.7 mmol/kg/day from the day of tumor implantation). Open symbols represent the tumor volumes of mice administered vehicle (same volume and duration as drug-treated animals). Each point and error bar represents the mean and SD of three tumors.

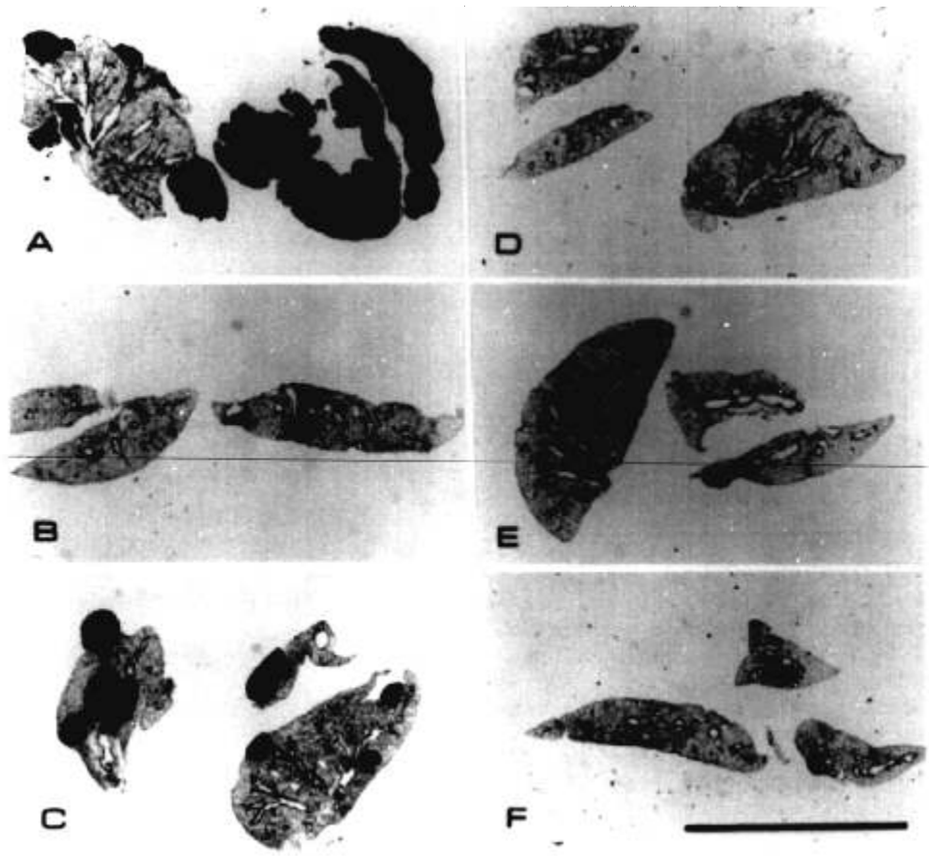


**Figure 2.** Effect of thalidomide on the development of lung metastases. Panels (a)–(c) are H&E histological sections prepared from the lungs of mice receiving vehicle. Panels (d)–(f) are similar sections from mice receiving thalidomide (0.7 mmol/kg/day from the day of tumor implantation). Sections show the formation of metastatic deposits (darkly stained areas). Sections were chosen to exhibit maximum area of metastases. Those sections showing no metastases indicate lungs which were metastases free. Bar = 1 cm.

Figure 3 shows the results of this study. The LL tumors from animals dosed with thalidomide were significantly more resistant to radiation than the control tumors from mice treated with vehicle only; however, no such radioprotection was seen in SCCVII tumors. In other experiments we examined the effect of thalidomide administration on the antitumor activity of tirapazamine combined with radiation in the LL tumors. An increased proportion of hypoxic cells within the tumor should enhance the effectiveness of tirapazamine. In these experiments thalidomide was administered daily (0.77 mmol/kg/day) for 5 days prior to treatment. Tirapazamine was administered (0.3 mmol/kg) immediately after an X-ray dose of 12.5 Gy, 2 h later the tumors were excised, a single cell suspension made and cells plated for colony formation. Figure 4 shows the results of this experiment, illustrating that tirapazamine potentiates the antitumor effectiveness of 12.5 Gy X-rays. The pretreatment with thalidomide further enhances this antitumor effect, though not to a statistically significant extent.

## Discussion

Anti-angiogenic therapy for cancer holds promise for clinical application because in adults neovascularization occurs rarely in non-pathologic tissue. Thalidomide exhibits anti-angiogenic activity when tested using the rat corneal micropocket assay. This prompted us to evaluate its activity in murine tumors. Thalidomide was used clinically in thousands of patients in the late 1950s and early 1960s and, with the important exception of embryotoxicity, is a relatively safe drug. A recent clinical

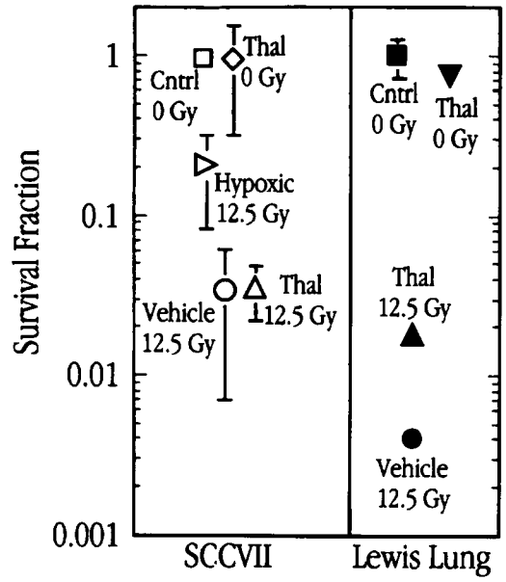


**Figure 3.** Effect of thalidomide (Thal) on the cell survival of SCCVII tumors (open symbols) and LL tumors (closed symbols) after irradiation of the tumor *in vivo* with 12.5 Gy. The absolute survival of SCCVII and LL tumors after 12.5 Gy differs because of known differences in the hypoxic fraction of the two tumors. Each point and error bar represents the mean and SD of three tumors, when error bars are absent the errors were smaller than the size of the point.

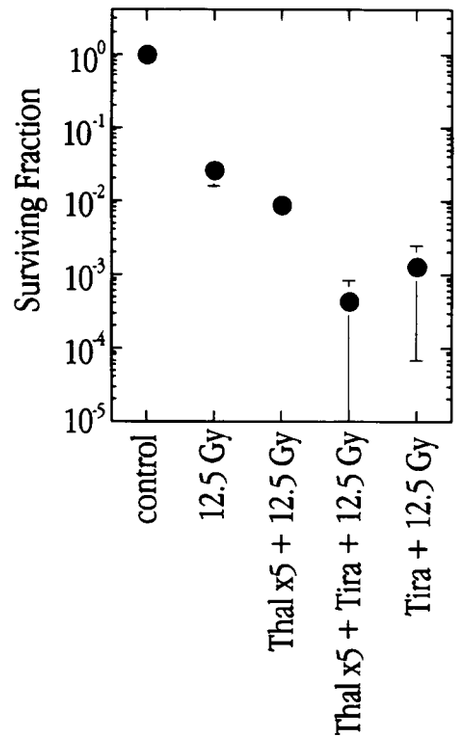


**Figure 4.** Effect of thalidomide (Thal) on the cell survival of LL tumors after *in vivo* treatment with 12.5 Gy X-rays and tirapazamine (Tira). Each point and error bar represents the mean and SD of three tumors. The calculation of surviving fraction of treatment involving thalidomide and tirapazamine takes into account cell loss. This is done by multiplying the surviving fraction by the tumor cell yield expressed as a fraction of the control tumor cell yield.

trial of thalidomide used to treat chronic graft versus host disease[17] used doses up to 1 g/day with minor side-effects.

Our studies showed that thalidomide had no significant effect on the growth of two primary murine tumors used in this study, and considering its prior clinical use and the absence of reports of angiogenesis-related toxicity this is not surprising. Thalidomide does appear to decrease tumor oxygenation since it rendered LL tumors more radioresistant and resulted in an enhancement of the activity against SCCVII tumors of combined radiation/tirapazamine treatment. The inhibition of metastases formed from transplanted LL tumors but the lack of activity against the primary tumor is paradoxical and may indicate that thalidomide inhibits an early stage in the neovascularization process of tumors that does not occur when a large number of cells are inoculated s.c. in order to induce 'primary' tumor development. Alternatively, thalidomide may affect LL metastases formation via some other mechanism.[18]

Our findings suggest that thalidomide may influence tumor pathophysiology via an anti-angiogenic mechanism, decreasing tumor oxygenation and altering metastatic potential. Further studies are warrented to more fully characterize the ability of thalidomide and its analogs to act as anti-angiogenic anti-cancer agents.

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