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Evaluation of N-(5-indanylsulfonyl)-N′-(4-chlorophenyl)-urea against xenografts of pediatric rhabdomyosarcoma*

Peter J. Houghton[1], Janet A. Houghton[1], Leann Myers[1], Pamela Cheshire[1], J. Jeffry Howbert[2], and Gerald B. Grindey[2]

[1]Laboratories for Developmental Therapeutics, Department of Biochemical and Clinical Pharmacology, St. Jude Childrens Research Hospital, Memphis, TN 38101, USA
[2]Eli Lilly and Company, Indianapolis, Indiana, USA

Summary. N-(5-indanylsulfonyl)-N′-(4-chlorophenyl)-urea (LY186641), a novel anticancer compound, was evaluated against six lines of rhabdomyosarcoma xenografts, each of which was established from tissue biopsies from untreated patients, and additional sublines selected as xenografts for primary resistance to vincristine, melphalan, and ifosfamide. LY186641 was given by oral gavage twice daily for 10 consecutive days or as 5-day courses repeated at 7-day intervals. At the optimal schedule, complete regressions of advanced tumors were obtained in each of the six rhabdomyosarcoma lines. There was no apparent cross-resistance in RMS lines selected for vincristine resistance or against multiple-drug-resistance KB cells in vitro. There was slight cross-resistance in xenografts selected for melphalan resistance, but not in an ifosfamide-resistant line. These results indicate that LY186641 may have significant clinical activity in the treatment of childhood rhabdomyosarcoma.

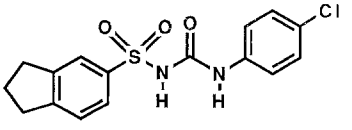
Introduction

Developing new agents that have significant activity in treating solid childhood tumors presents problems rarely encountered in the more common adult neoplasms. This is partly due to the relatively small number of patients, which restricts the rate at which new compounds may be evaluated. Furthermore, where effective and potentially curative therapy is available, efficacy trials (phase II) are conducted in relapsed patients who have failed multiple therapeutic modalities. Thus, tumors may be highly resistant and patients may have reduced tolerance to a new agent. Therefore, it is probable that an agent demonstrating little activity in phase II evaluation may have a significant effect on the same tumor type at diagnosis; we have recently demonstrated this for L-phenylalanine mustard (melphalan [7]). In preclinical tests using xenografts of childhood rhabdomyosarcoma (RMS) melphalan caused significant regressions in five of six tumor lines, suggesting a response rate of approximately 80% [12]. Indeed, this was found when melphalan (given at standard dose levels) was evaluated against advanced-stage RMS at diagnosis. In contrast, in a phase II trial against refractory RMS, only 1 of 13 patients had a partial response [7].

These results have, in part, validated the use of a panel of RMS xenografts in representing a preclinical phase II situation for previously untreated disease. We therefore used these models to identify new agents that may have significant activity against RMS and to prioritize their clinical evaluation in childhood cancer.

Most regimens for treatment of childhood solid tumors incorporate vincristine, doxorubicin, actinomycin D, and an epipodophyllotoxin [16, 17]. Each of these agents has been associated with a multiple-drug-resistance (MDR) phenotype conferred by a putative efflux pump [15]. Thus, one criterion for evaluating a new agent in depth is that it not be cross-resistant with this typical MDR phenotype. Also of interest are compounds that have minimal marrow-suppressive activity, as many protocols used for treatment of childhood malignancy are highly toxic to marrow.

One novel agent currently in phase I evaluation in adults is N-(5-indanylsulfonyl)-N′-(4-chlorophenyl)-urea (LY186641; Fig. 1). This compound has significant activity against murine solid tumors [6] and human xenografts of epithelial origin [5]. Clinically it has little marrow toxicity. Thus, it fulfilled certain criteria that made this agent with a novel chemical structure interesting to evaluate against childhood solid tumors in a preclinical model. We report the significant therapeutic activity of N-(5-indanylsulfonyl)-N′-(4-chlorophenyl)-urea against advanced-stage xenografts of RMS.



LY186641

Fig. 1. Chemical structure of N-(5-indanylsulfonyl)-N′-(4-chlorophenyl)-urea

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Abbreviations: RMS, rhabdomyosarcoma; L-PAM, melphalan, L-phenylalanine mustard; ifos, ifosfamide; VCR, vincristine

Offprint requests to: P. J. Houghton

Table 1. Responsiveness of xenografts of childhood rhabdomyosarcoma

Tumor	Dose (mg/kg): 300	200	100
Schedule, b. i. d. × 10 days:			
Rh12		++++[a]	+++
Rh12/VCR-3		++++	ND
Rh12/ifos		++++	ND
Rh18		++++	ND
Rh18/VCR-3		+++++	ND
Rh18/L-PAM		+++	ND
Rh28		+++++	+++++
Rh28/L-PAM		+++++	+++
IRS68		++++	ND
Schedule, (b.i.d. × 5 days)$_2$[b]:			
Rh12		++++	+++
Rh30	++++++	+++++	ND
IRS49		+++++	+++++
Rh18/VCR-3		+++++	+++++
IRS68	+++++	+++++	ND
Schedule, (b.i.d. × 5 days)$_3$[b]:			
Rh18		+++++	+++++
Rh12		+++++	++++

[a] Tumor response criteria: +++, growth inhibition of ≥3 Td_2; ++++, growth inhibition of >3 × Td_2 plus volume regression of ≥50%, +++++, complete regression with subsequent regrowth; ++++++, complete regression with no growth during the period of observation (≥84 days). (Td_2, mean time for tumor volume to double)
[b] Subscript indicates the number of courses
ND, not determined

Materials and methods

In vitro studies. Cell lines KB3-1 and KBCh^{R}8-5 were obtained from Dr. I. Pastan and maintained in antibiotic-free medium containing 10% fetal calf serum (Gibco). KBCh^{R}8-5 was grown in the presence of 10 ng/ml colchicine [1] and overexpresses the *mdr*1 gene 16-fold relative to the parental KB3-1 clone. For drug sensitivity studies, cells were seeded at 2 10^5/cm^2 and, 24 h later, continuously exposed to cytotoxic agents for 72 h. Cell number was determined by counting nuclei after cell lysis [2].

Immune deprivation of mice. Female CBA/CaJ mice (Jackson Lab, Bar Harbor, Me), 4 weeks of age, were immune-deprived by thymectomy, followed 3 weeks later by whole-body irradiation (950 cCy) using a ^{137}Cs source. Mice received 3×10^6 nucleated bone marrow cells within 6–8 h of irradiation [9].

Tumor lines. Four of the six independently derived lines from previously untreated RMS have been described [10, 11]. Two additional lines, IRS-49 (alveolar) and IRS-68 (embryonal), were established from tissues obtained through the Intergroup Rhabdomyosarcoma Study (designated IRS). Both were used as early passage material in which "minimal deviation" from the patient would be anticipated. For chemotherapy studies, all tumors were used within 20 passages of their engraftment in mice. Each tumor grows routinely in over 90% of recipient mice, and all are human as determined by karyotype and species-specific isoenzyme patterns. The chemosensitivity of these lines has previously been reported for conventional agents in the therapy of RMS [11] as well as for L-PAM [12]. Sublines of Rh12, Rh18, and Rh28 that were selected *in situ* for resistance to vincristine (Rh12/VCR-3, Rh18/VCR-3), melphalan (Rh18/L-PAM, Rh28/L-PAM), and ifosfamide (Rh12/ Ifos) have previously been described [8, 13, 14].

Growth inhibition studies. Mice bearing bilateral subcutaneous tumors received the agent when tumors were ⩾1 cm in diameter. Tumor response was determined at 7-day intervals using digital calipers (maxcal) interfaced through an RS 232 to a Suntronics AT microcomputer. Two perpendicular diameters were used to compute volumes [12]. Growth delay was calculated from the difference in days required for treated tumors to grow to 4 times their volume at the start of treatment compared with vehicle-treated controls. For each treatment group, six or seven tumor-bearing mice were used. Relative tumor volumes were calculated from the formula RTV = (V_x/V_o), where V_x = the volume on day X and V_0 is the volume of tumor at the initiation of treatment. To equate responses in tumor lines

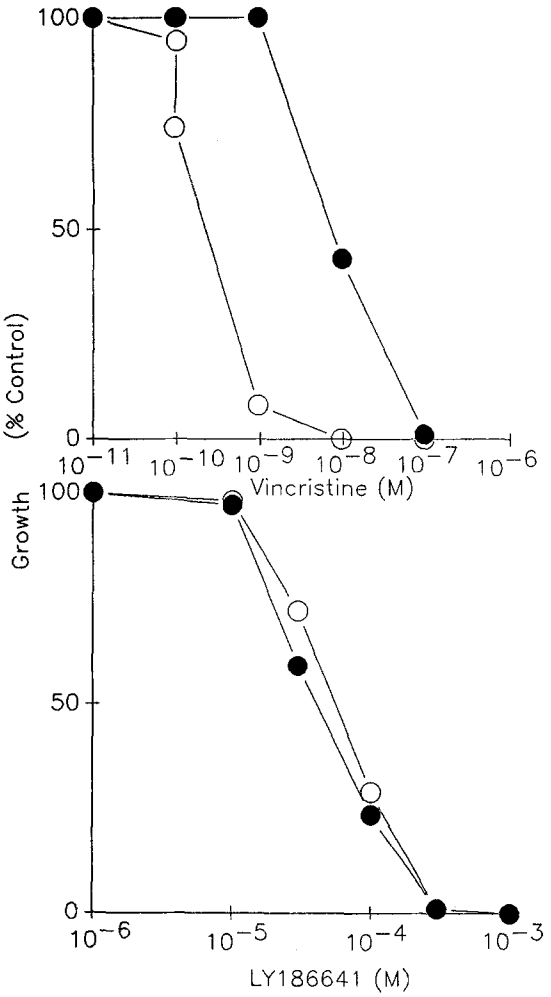

Fig. 2. Sensitivity of KB3-1 (○) and KBCh^{R}8-5 (●) cells exposed for 72 h to vincristine (*top*) or LY186641 (*bottom*). Each point represents the mean of triplicate determinations

Table 2. Toxicity of LY188641 using different schedules of administration

Schedule	Dose (mg/kg)	Deaths[a]/total
b. i. d. × 10 days	Emulphor	5/62
	200	9/84
(b. i. d. × 5 days)$_2$	300	0/14
	200	0/35
(b. i. d. × 5 days)$_3$	200	0/14

[a] Death within 21 days of ending therapy

that demonstrate different rates of growth, inhibition was normalized by expressing this as a function of tumor volume-doubling time. Mean volume-doubling times during exponential growth for Rh12, Rh18, Rh28, Rh30, IRS-49, and IRS-68 tumors were 7.9, 6.3, 9.9, 9.3, 15.5, and 6.4 days, respectively. For L-PAM-resistant sublines of Rh18 and Rh28, doubling times were 4.2 and 10.1 days, respectively. Grading of tumor responses is given in Table 1; the definition of $\geq 50\%$ regression required that each tumor within a group demonstrate such a reduction in volume at some point after treatment.

Formulation and administration. LY186641 was suspended in 5% Emulphor EL-620 (GAF Corp., Wayne, NJ) and given by oral gavage (0.1 ml/20 g body weight) after vigorous resuspension of the material. All protocols used twice-daily administration (b. i. d.), the doses being spaced by 8 h.

Statistical analysis. The results of individual studies were evaluated with one-way analysis of variance, using the number of days to reach 4 times the original tumor volume as the dependent variable. Only tumors from mice that survived the entire study were included in the analyses, and any tumor that did not reach 4 times its original volume was assigned a default value of the maximal duration of the study. To compare the efficacy of various courses of treatment, data were collapsed across studies within a tumor line. The percentage of tumors showing partial and/or complete regression and any regrowth were calculated for the individual tumor lines.

Results

In vitro studies

One criteria that we have used to prioritize *in vivo* evaluation is that a new agent should be equally active against cells that exhibit a typical MDR phenotype conferred by the *mdr*1 gene product. As shown in Fig. 2, KBCh^{R}8-5 was approximately 40-fold resistant to VCR compared with the parental KB3-1 cell line. In contrast, LY186641 had equal potency against both cell lines.

In vivo studies

LY186641 was given b. i. d. for 10 days to mice bearing advanced xenografts of RMS that were derived from previously untreated patients (Rh12, Rh18, Rh28, and IRS-68). At a dose of 200 mg/kg, there were 9/84 deaths within 21 days of the end of treatment (Table 2), as well as significant growth inhibition ($P < 0.001$) and $> 50\%$ volume regressions in all tumor lines (Fig. 3). Complete volume regression with no regrowth was determined in Rh28 xenografts (Table 1).

On the 10-day schedule there was some toxicity in control groups that received vehicle alone (5% emulphor, b. i. d. 10 days p. o.), where 5/62 deaths occurred. To reduce toxicity, which may have been partly mechanical in origin, LY186641 was given b. i. d. for 5 days and the

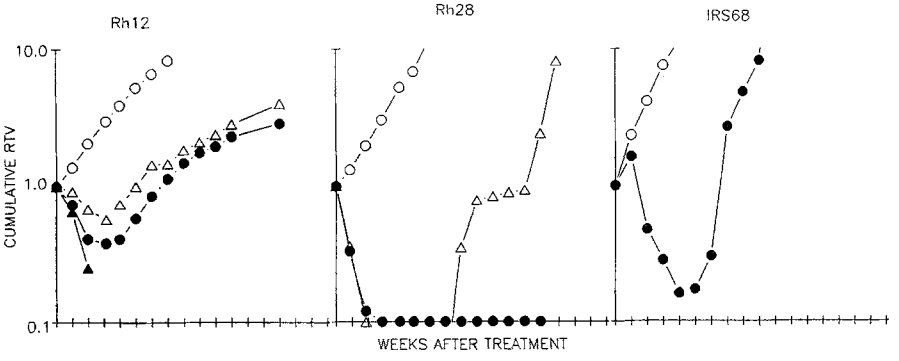

Fig. 3. Responses of rhabdomyosarcoma xenografts derived from untreated tumor biopsies. Each curve represents the mean growth for groups of 12 or 14 tumors in mice receiving 5% emulphor (○) or LY186641 at a dose of 400 (▲), 200 (●), or 100 (△) mg/kg. All mice received drug or vehicle (control) twice daily for 10 days. Relative tumor volumes were calculated as described in *Materials and methods*

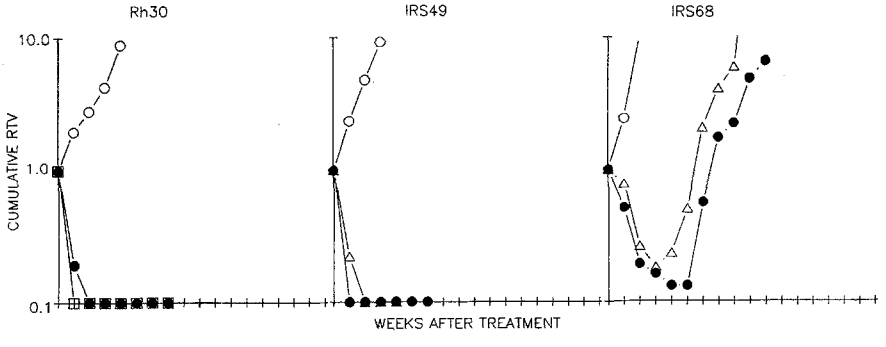

Fig. 4. Responses of xenografts in mice receiving LY186641 b. i. d. × 5 repeated at 7 days. Complete regression of advanced Rh30, IRS-49, and IRS-68 tumors was achieved. Each curve represents the mean relative tumor volume for 12 or 14 tumors/group. LY186641 was given at a dose of (□) 300, (●) 200, or (△) 100 mg/kg; (○) 5% emulphor

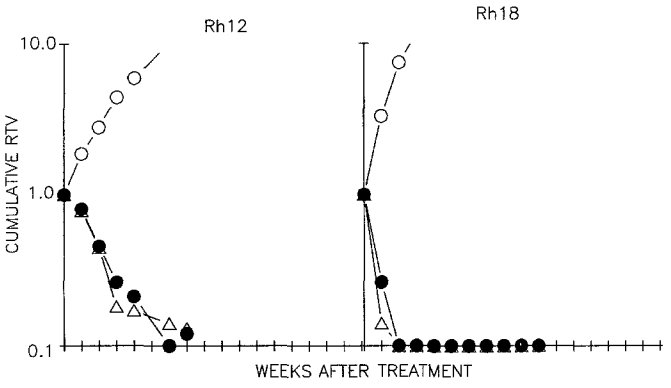

Fig. 5. Responses of Rh12 and Rh18 xenografts in mice receiving three 5-day courses of LY186641 over 19 days. Mice received 5% emulphor (○) or a dose of 200 (●) or 100 (△) mg/kg LY186641 b.i.d. ×5 days for three courses

course was repeated at 7-day intervals. At 200 mg/kg, two courses caused no deaths (0/35) and the dose could be increased to 300 mg/kg with no lethality (Table 2). Comparison of responses between the two schedules (Rh12, Rh18/VCR-3, and IRS-68) demonstrated that two courses of 5 days were as effective as the sequential 10-day course, but the former had reduced toxicity. On this schedule, complete regressions with no regrowth during the period of observation (84 days) were observed in Rh30, IRS-49, and Rh18/VCR-3. IRS-68 xenografts regressed completely but regrew during the period of observation. Using this schedule, 300 mg/kg was well tolerated (0/14 deaths); however, in IRS-68 tumor responses were similar to those at the 200 mg/kg level (Fig. 4). A third schedule, in which three courses (b.i.d. × 5 days) were given, showed greater activity against Rh12 and Rh18 xenografts (Fig. 5, Table 1). This schedule was well tolerated (0/14 deaths; Table 2) and caused complete regression of advanced Rh12 and Rh18 xenografts.

LY186641 was also evaluated against xenografts that exhibit low-level, stable resistance to ifosfamide (Rh12/Ifos), vincristine (Rh12/VCR-3; Rh18/VCR-3), and melphalan (Rh18/L-PAM; Rh28/L-PAM). There was no apparent cross-resistance in vincristine-selected lines (Table 1), and Rh18/VCR-3 was markedly more sensitive than Rh18 (Fig. 6). The sensitivity of Rh12 and its vincristine- and ifosfamide-resistant sublines was similar, but both Rh18/L-PAM and Rh28/L-PAM were less sensitive than the parental xenografts (Table 1; Fig. 6).

Discussion

N-(5-Indanylsufonyl)-N′-(4-chlorophenyl)-urea (LY-186641) exhibits a high therapeutic selectivity against both murine tumors and human tumor xenografts. We extended studies to xenografts of childhood solid tumors, and the data presented show very significant activity against RMS. Using optimal scheduling, complete regressions were obtained in each of six lines derived from previously untreated tumor material. Thus, in this model LY186641 demonstrates activity at least similar to that of vincristine and melphalan, two agents with very significant activity against childhood RMS [4, 7].

In selecting a new compound prior to extensive testing *in vivo*, one criterion is that resistance does not segregate with the typical MDR phenotype. Although at present the clinical significance of accelerated drug efflux via the *mdr*1 gene product (GP-170) is unknown, overexpression of *mdr*1 in childhood tumors has been reported [3]. Virtually all childhood tumors are treated with drugs associated with the MDR phenotype (vincristine, actinomycin D, doxorubicin, and possibly VP-16). Hence, it is probable that a new agent would demonstrate marginal activity in a phase II evaluation, where resistance may be mediated by this mechanism, and would generate insufficient enthusiasm for further development. Clearly, LY186641 was equally effective against $KBCh^R8$-5 and parental KB3-1 cells *in vitro*, hence fulfilling criteria for subsequent *in vivo* evaluation. Against xenografts selected *in vivo* for resistance to vincristine, LY186641 either showed similar activity or was more active (Rh18/VCR-3). Some cross-resistance was observed in lines selected for L-PAM resistance (Fig. 5), but not in an ifosfamide-selected line (Rh12/Ifos) (Table 1).

Administration of LY186641 for 10 consecutive days caused lethality in approximately 10% of tumor-bearing mice. However, administration of 5% emulphor (control vehicle) also resulted in some deaths, suggesting that lethality may have been related to p.o. dosing b.i.d. Toxicity was reduced where two 5-day courses were given over 12 days, with no reduction in antitumor effect. Using this schedule, doses of 300 mg/kg for two courses or 200 mg/kg for three courses were well tolerated.

LY186641 administration for 10 consecutive days also resulted in complete regression with no regrowth in advanced Rh18/VCR-3, Rh28, and IRS49 xenografts. Complete regression with subsequent regrowth was achieved in IRS68 tumors. Two lines, Rh12 and Rh18, were less responsive, and only partial regressions were achieved. Furthermore, in Rh12 responses were similar at doses of

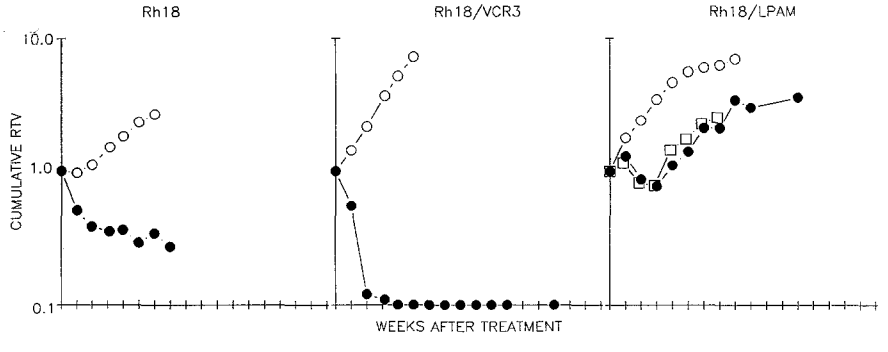

Fig. 6. Responses of Rh18 xenografts and of sublines selected for resistance to vincristine (Rh18/VCR-3) or melphalan (Rh18/L-PAM). Each curve represents the mean response of 12 or 14 xenografts per group. Mice received 5% emulphor (○) or a dose of 300 (□) or 200 (●) mg/kg LY186641 b.i.d. ×10 days

200 and 100 mg/kg. It was thus of interest to determine whether greater efficacy could be achieved by extending the duration of administration. Three courses of LY186641 were given over 19 days. This prolonged schedule of administration appeared to be more efficacious against Rh18 xenografts. For example, treatment for 10 consecutive days (dose 200 mg/kg) caused no complete regressions, whereas 100% complete regressions were obtained after three 5-day courses at doses of 200 and 100 mg/kg. Thus, at a lower total dose of LY186641, greater tumor responses were obtained using the prolonged schedule of administration. Similar results were obtained in Rh12 tumors, where three courses of therapy gave somewhat better tumor responses than those obtained after two courses.

In summary, LY186641 demonstrates significant activity against a comprehensive model of childhood RMS. In this model, activity was similar to that produced by vincristine and melphalan, two of the most effective agents used for treating RMS in children. Data obtained in the present xenograft models suggest that this novel agent may have value in the treatment of childhood RMS.

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