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Preclinical study

Significant activity of a novel cytotoxic agent, LY295501, against a wide range of tumors in the human tumor cloning system

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The diarylsulfonylureas (DSUs) are a novel class of cytotoxic agents with high potential for activity. LY295501 is one of the most active DSUs. In this study, we evaluated the cytotoxicity of LY295501 utilizing the human tumor cloning assay. LY295501 was tested at 10, 50 and 100 μg/ml using either 1 h or continuous exposure schedules. The majority of common solid tumors were evaluated including breast, colorectal, non-small cell lung and ovarian carcinomas. LY295501 demonstrated significant activity against all tumor types tested. Antitumor activity was noted after either 1 h or continuous exposure schedules at all concentrations tested. A concentration–response relationship was noted, with increasing concentrations of LY295501 leading to more cytotoxicity. Cytotoxicity, on the continuous exposure schedule, was noted in 38% of tumors exposed to LY295501 at 10 μg/ml, 58% of tumors exposed at 50 μg/ml (p=0.002 for 10 versus 50 μg/ml) and 72% of tumors exposed at 100 μg/ml (p=0.008 for 50 versus 100 μg/ml). In addition, more cytotoxicity was observed on the continuous exposure schedule compared to the 1 h schedule at all concentrations tested ($p < 0.01$). The substantial activity of LY295501 in the human tumor cloning assay coupled with its clinical activity in phase I studies supports further clinical development of this agent. [© 1999 Lippincott Williams & Wilkins.]

Key words: Diarylsulfonylurea, human tumor cloning, system, LY295501.

Introduction

The diarylsulfonylureas (DSUs) are a novel class of cytotoxic agents with high potential as anticancer drugs.[1,2] The mechanism of action of DSUs is unknown. There is no inhibition of nucleic acids or protein synthesis.[3] A first-generation compound, sulofenur (LY186641), underwent clinical testing in phase I and II studies.[4–8] In spite of some activity in phase I studies,[9,10] sulofenur demonstrated a low level of activity in phase II studies.[8,11] This low level of activity of sulofenur coupled with its unusual toxicities (methemoglobinemia, anemia and hemolysis) did not justify further clinical development of this compound.[5,6,12,13] Further efforts were directed toward the development of second-generation compounds that might have a better therapeutic index. Based on structure-activity studies, LY295501 was identified as having a different side effect profile compared to sulofenur.[2] In addition, preclinical evaluation indicated that it has significant antitumor activity.[1]

LY295501 has significant activity against a broad spectrum of human tumor xenografts grown in immunologically deficient mice including colon (CX-1, HC-1, HXGC3 and VRC5), lung (LX-1), mammary (MX-1) and pancreas (PaCa-2 and BxPc-3) xenograft tumor models.[1,14,15] Because DSUs, including LY295501, bind extensively to plasma proteins,[16] *in vitro* cytotoxicity studies were done with cells grown in serum-free UltraCHO medium. In this medium, LY295501 is one of the most active cytotoxic DSUs. The IC_{50} against the CCRF-CEM leukemia cell line after 72 h exposure is 0.18 ± 0.03 mM. By comparison, sulofenur is 6 times less potent with an IC_{50} of 1.1 ± 0.1 mM.[15]

The objectives of this study were to evaluate the cytotoxicity of LY295501 using different concentrations and two different schedules of exposure utilizing the human tumor cloning assay—a useful system to screen for antitumor activity of new cytotoxic compounds.

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Materials and methods

After obtaining informed consent, malignant effusions, ascites and bone marrow aspirates containing tumor cells, as well as solid tumor specimens were collected from patients undergoing procedures done as part of their diagnostic work-up or as part of treatment for their disease. No samples were obtained solely for research purposes. Solid tumors or lymph nodes were minced into 2–5 mm fragments in the operating room and immediately placed in McCoys medium 5A plus 10% heat-inactivated new-born calf serum and 1% penicillin/streptomycin. Within 4 h, these solid tumors were mechanically disassociated with scissors, forced through no. 100 stainless steel mesh, through 25 gauge needles and then washed with McCoys medium as previously described.[17–21] Ascitic, pleural, pericardial fluids and bone marrows were obtained by standard techniques. The fluid or marrow was placed in sterile containers containing 10 U of preservative-free heparin/ml of malignant fluid or marrow. After centrifugation at 150 *g* for 10 min, the cells were harvested and washed with McCoys plus 10% heat-inactivated fetal calf serum. The viability of cell suspensions was determined on a hemocytometer with Trypan blue. The final concentrations of LY295501 tested were 10, 50 and 100 μg/ml at both 1 h and continuous exposures.

For the 1 h exposure studies, the cells were incubated for 1 h with LY295501 in McCoys medium, then washed to simulate the disappearance of the drug from the body. Cells were suspended in 0.3% agar in enriched CMRL 1066 supplemented with 15% heat-inactivated horse serum, penicillin (100 U/ml), streptomycin (2 mg/ml), glutamine (2 mM), insulin (3 U/ml), asparagine (0.6 mg/ml) and HEPES buffer (2 mM). Cells were plated in 35 mm Petri dishes in a top layer of agar over an underlayer of 0.3% agar to prevent growth of fibroblasts. Three plates were prepared for each data point. For the continuous exposure, cells were combined with LY295501 in the CMRL medium described above, then plated as for the 1 h exposure. It should be noted that the 2 week exposure only means that the compound was not washed from the culture during the 2-week incubation period (additional drug was not added to the culture). The plates were placed in a 37 C incubator and removed on day 14 for counting of the number of colonies in each plate. The numbers of colonies (defined as more than 50 cells) formed in the three compound-treated plates were compared to the numbers of colonies formed in the three untreated control plates and the percent colonies surviving at each concentration was calculated.

To assure the presence of an excellent single-cell suspension on the day of plating, positive controls were used. For each tumor tissue sample tested, three positive control plates were set up to contain the cell poison orthosodium vanadate at 200 μg/ml.[22] This positive control agent (orthosodium vanadate) should destroy all clonogenic cells. If there was no effect of positive control on colony formation, then the single-cell suspension on day 0 was poor (since orthosodium vanadate does not affect clumps) and the tumor sample test was considered non-evaluable.

A test is defined as an experiment, performed on a unique tumor tissue sample, that contains untreated control, positive control and three specified compound concentration levels. An evaluable test is one having an average of 20 or more colonies present on day 14 in the untreated control plates and less than 30% survival in the positive control (orthosodium vanadate) plates when compared to untreated control plates. A response is defined as a 50% or greater decrease in colony formation compared with the control. This cut-off was found useful in predicting patient response using the human cloning assay.[23] Statistical analyses were performed using NcNemars test for paired proportions.

Results

Freshly explanted tumor specimens were exposed to LY295501 using either 1 h or continuous exposure schedules. The number of specimens tested and the number of assessable specimens per tumor-type is listed in Tables 1 and 2, on the 1 h and continuous exposure schedules, respectively. It was found that 43 and 35% of the specimens were assessable for cytotoxicity on the 1 h and continuous exposure schedules, respectively. The tumor types tested included the majority of common solid tumors including breast, colorectal, non-small cell lung and ovarian tumors.

Antitumor activity was noted after 1 h exposure and continuous exposure at all concentrations tested (Tables 1 and 2). A concentration-response relationship was noted, with increasing concentrations leading to more cytotoxicity. This concentration-response relationship was observed on both the 1 h and continuous exposure schedules. On the 1 h schedule, cytotoxicity was noted in 10% of tumors treated at 10 and 50 μg/ml, and in 25% of tumors treated at 100 μg/ml (p=0.083). Cytotoxicity was noted in 38% of tumors exposed to LY295501 at a concentration of 10 μg/ml, 58% of tumors exposed to LY295501 at a concentration of 50 μg/ml (p=0.002 for 10 versus 50 μg/ml) and in

72% of tumors exposed to LY295501 at a concentration of 100 μg/ml (p=0.008 for 50 versus 100 μg/ml).

In addition, more cytotoxicity was observed on the continuous exposure schedule compared to the 1 h schedule at all concentrations tested. At concentrations of 10 μg/ml, cytotoxicity was noted in 38% of tumors exposed continuously compared to 10% of tumors exposed to the 1 h schedule (p=0.025 for 13 tumors tested on both schedules). At concentrations of 50 μg/ml cytotoxicity was noted in 58% of tumors on the continuous exposure schedule compared to 10% of tumors on the 1 h schedule (p=0.008 for 13 tumors tested on both schedules). Similarly, at concentrations of 100 μg/ml, cytotoxicity was noted in 72% of tumors exposed continuously compared to 25% of tumors exposed to the 1 h schedule (p=0.003 for 13 tumors tested on both schedules).

LY295501 demonstrated significant activity against all tumor types tested (excluding pancreas with only one pancreas tumor assessable for cytotoxicity). The level of activity was over 60% against gastric, colon, non-small cell lung, melanoma and ovarian carcinoma among others (Table 2). This level of activity was observed at concentrations that are easily achievable in patients.[26]

Discussion

LY295501 represents a novel class of cytotoxic agents with potential activity against a wide range of solid tumors. In this study, the activity of LY295501 against freshly explanted human tumors in the tumor colony-forming units assay has been evaluated on either 1 h or

Table 1. *In vitro* antitumor activity of LY295501 on the 1 h exposure schedule

Tumor type	No. of specimens attempted/assessable/inhibited (% inhibited/assessable)					
	10 μg/ml		50 μg/ml		100 μg/ml	
Breast	1/1/0	(0)	1/1/0	(0)	1/1/0	N/A
Colorectal	7/3/1	(33)	8/3/1	(33)	7/3/1	(33)
Gastric	1/0/0	N/A	1/0/0	N/A	1/0/0	N/A
Melanoma	7/4/0	(0)	7/4/0	(0)	7/4/1	(25)
Mesothelioma	5/0/0	N/A	5/0/0	N/A	5/0/0	N/A
Non-small cell lung	1/0/0	N/A	1/0/0	N/A	1/0/0	N/A
Ovarian	9/8/1	(13)	9/8/0	(0)	9/8/2	(25)
Pancreas	2/0/0	N/A	2/0/0	N/A	2/0/0	N/A
Renal cell	4/1/0	(0)	5/1/0	(0)	4/1/0	(0)
Unknown primary	3/1/0	(0)	2/1/1	(100)	2/1/1	(100)
Other	7/2/0		8/3/0	(0)	7/2/0	(0)
Total	47/20/2	(10)	49/21/2	(10)	46/20/5	(25)

Table 2. *In vitro* antitumor activity of LY295501 on the continuous exposure schedule

Tumor type	No. of specimens attempted/assessable/inhibited (% inhibited/assessable)					
	10 μg/ml		50 μg/ml		100 μg/ml	
Breast	7/5/2	(40)	8/5/1	(20)	7/5/1	(20)
Colorectal	16/6/0	(0)	16/6/3	(50)	16/6/4	(67)
Gastric	5/2/1	(50)	5/2/2	(100)	5/2/2	(100)
Melanoma	17/8/3	(38)	17/8/5	(63)	17/8/5	(63)
Mesothelioma	6/1/0	(0)	6/1/0	(0)	6/1/1	(100)
Non-small cell lung	6/3/2	(67)	6/3/2	(67)	6/3/2	(67)
Ovarian	38/16/9	(56)	39/16/11	(69)	38/16/14	(88)
Pancreas	8/1/0	(0)	8/1/0	(0)	8/1/0	(0)
Renal cell	8/3/0	(0)	9/3/2	(67)	8/3/3	(100)
Unknown primary	15/0/0	N/A	15/0/0	N/A	15/0/0	N/A
Other	24/8/3	(38)	24/8/5	(63)	24/8/6	(75)
Total	150/53/20	(38)	153/53/31	(58)	150/53/38	(72)

continuous exposure schedules. Although activity has been documented on both schedules, the continuous exposure schedule demonstrated higher levels of activity at all doses tested. Due to the long half-life of LY295501 in man, with an average of 136 h (range: 43–300) as estimated in phase I studies,[26] the continuous exposure schedule rather than the short 1 h exposure more accurately represents exposure in man.

The activity of LY295501 was concentration-related on either the 1 h or continuous exposure schedules. The highest concentration of 100 μg/ml is achievable in patients for extended periods of time, suggesting that effective cytotoxic levels are feasible.[26]

The activity of LY295501 in this model was against a broad range of solid tumors including renal cell carcinoma, non-small cell lung carcinoma, gastric carcinoma and ovarian carcinoma. To date, clinical activity in phase I studies was observed in non-small cell lung carcinoma and renal cell carcinoma.[24–26] This broad range of activity in this model coupled with its clinical activity makes this compound very promising.

Since LY295501 binds extensively to plasma proteins[16] and since the culture media in the cloning assay contains only 15% serum, it is possible that a higher concentration of the free fraction of LY295501 is achievable in the cloning system than in patients. However, the activity noted in phase I studies suggests that cytotoxic levels of free drug are achievable in spite of extensive protein-binding.

In summary, LY295501 is a novel and very promising cytotoxic agent with high activity against a wide range of solid tumors at concentrations that are easily achievable in man. Based on its *in vitro* activity and the clinical activity observed in phase I studies, LY295501 should be evaluated for activity in a variety of solid tumors in phase II studies.

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