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Pyran Copolymer as an Effective Adjuvant to Chemotherapy against a Murine Leukemia and Solid Tumor

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SUMMARY

Pyran (divinyl ether-maleic anhydride) copolymer (NSC 46015) is a polyanion with interferon-inducing and macrophage-stimulating properties, and therefore it has been studied as a possible antitumor agent. Extensive studies using pyran as an adjuvant to chemotherapy against the LSTRA murine leukemia and the Lewis lung carcinoma were performed. Pyran was effective over a dose range of 0.1 to 100 mg/kg/day. Single and multiple dose schedules were both capable of producing significant numbers of cures or increasing life-span, but pyran was ineffective if used without remission inducing chemotherapy.

Various molecular weights of pyran copolymer were compared against NSC 46015 for adjuvant activity as well. In general, NSC 46015 tended to be the most efficacious, but all the pyran sizes that were tested possessed significant activity.

INTRODUCTION

Pyran is a negatively charged copolymer of divinyl ether and maleic anhydride. It can be synthesized in chains of varying length, but the most frequently studied preparation is NSC 46015, which was designed for clinical trials. After its ability to induce interferon (9, 10) and protect against oncogenic virus infection (5, 18) was established, pyran was found to have antitumor activity (12, 15, 20). These important activities seem to be primarily mediated through macrophage stimulation (6, 7, 19) and also possibly B-cell stimulation to form antibody (1). An additional property of NSC 46015, and several of its analogs, is its ability to inhibit the RNA-dependent DNA polymerase of the oncogenic avian myeloblastosis virus (13). Because pyran seemed to be a highly immunoactive compound, it was believed that it might be useful as an immunoadjuvant to effective antitumor therapy using conventional chemotherapeutic drugs. Since pyran was found to be toxic in early clinical trials (12), careful dose and regimen studies were done so that clinical toxicity might be eliminated while retaining the beneficial effects. Also, various sizes of pyran were tested for significant adjuvant activity without drug toxicity. The results provide evidence that pyran is indeed effective as an adjuvant to chemotherapy and that it will not lose this activity when used in a way that should minimize patient toxicity.

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MATERIALS AND METHODS

Tumor. The LSTRA murine leukemia was initially established in this laboratory by inoculation of BALB/c mice with Moloney leukemia virus. The leukemia has been carried in the transplantable ascites form in BALB/c × DBA/2 F_1 (hereafter called $CD2F_1$) mice for over 150 generations. The Lewis lung carcinoma has been carried as a transplantable solid tumor in C57BL/6J mice in our laboratory for over 80 generations. Cell counts were performed using trypan blue exclusion to determine viability, and the cells were suspended in Eagle's minimal essential media. Both tumors were injected s.c. in the right inguinal region of the mouse.

Mice. C57BL/6J × DBA/2 F_1 (hereafter called $B6D2F_1$) and $CD2F_1$ mice were obtained from the Mammalian Genetics and Animal Production Section of the NIH, Bethesda, Md. All animals were 6 to 8 weeks of age and weighed approximately 25 g when used in experiments. The animals were housed in plastic cages with air filter bonnets and were given tap water and Purina laboratory chow *ad libitum*.

Drug. BCNU and 1-(2-chloroethyl)-3-(*trans*-4-methylcyclohexyl)-1-nitrosourea (NSC 95441) were supplied by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute, NIH, Bethesda, Md. Both drugs were suspended in a 0.3% solution of hydroxypropyl cellulose (Klucel) and given s.c. in an injection volume of 1% body weight. Pyran copolymer (NSC 46015) and pyrans of different viscosities were kindly supplied by Dr. David Breslow of Hercules Research Center, Wilmington, Del. All pyrans were dissolved in 0.9% NaCl solution, adjusted to pH 7.0 using NaOH, and were given i.p. using a mg/kg dosage regimen in an injection volume equal to 1% body weight.

RESULTS

Previous experiments in our laboratory showed that $CD2F_1$ mice inoculated with 1×10^4 LSTRA leukemia cells all died in approximately 13 to 15 days with systemic leukemia. Chemotherapy with BCNU[1] at a tolerated dose of 30 mg/kg administered on the 7th day after tumor inoculation, a time when systemic leukemia is apparent, resulted in a prolonged survival time of approximately 23 to

[1] The abbreviation used is: BCNU, 1,3-bis(2-chloroethyl)-1-nitrosourea (NSC 409962).

29 days (4, 14, 16, 17). Table 1 demonstrates that adjuvant therapy after BCNU with pyran copolymer was effective if treatment was started on day 13 or day 20. Daily or every-other-day therapy for a total of 3 doses produced 70 to 90% long-term survivors (cures) compared to chemotherapy alone which produced only 30%. Pyran started on Day 6 was no more effective than chemotherapy alone. The time period of 13 to 20 days corresponded to the chemotherapeutic-induced remission period at a time when the early suppressive effects of BCNU had diminished and the tumor load was still at a low level (3).

Having established the most effective time interval for pyran therapy following BCNU induction of remission, we determined the efficacy of a single dose of pyran as opposed to multiple treatments. Chart 1 demonstrates that a significant number of long-term survivors were produced by either a single dose of pyran on Day 13 or by multiple doses beginning on Day 13, 6 days after BCNU was given. Multiple treatments given every other day (Group 6) or every 3rd day (Group 7) were best, but only slightly more effective than single (Group 4) or daily (Group 5) treatments. In Group 2 pyran alone without BCNU therapy was no more effective than in the untreated controls (Group 1).

Since a single treatment of pyran after BCNU remission was capable of producing a large number of long-term survivors compared to chemotherapy alone, a dose study could be done utilizing only a single treatment. Table 2 summarizes the results of tests in which the dose of pyran was varied. Compared to BCNU therapy alone, pyran appeared to be effective as an adjuvant at doses ranging from 0.1 to 100 mg/kg. Pyran was ineffective at 200 mg/kg and was associated with 40% toxic deaths. There was no evidence of drug toxicity at the lower doses of pyran used.

With the knowledge that the original pyran copolymer (NSC 46015) was effective in a single treatment and over a wide-dose spectrum, many of the pyrans of different viscosities could be tested for comparative adjuvant activity. It had previously been shown that size was critical for the activity of polyinosinic-polycytidylic acid and polyacrylic acid against the L1210 leukemia (11) and, therefore, it was of interest to see whether this held true for pyrans of varying sizes. The intrinsic viscosities shown in Table 3 do not correlate directly with the molecular weight of the pyran polymer. Rather, they are a relative measure, indicating that the average molecular weight of the compound under consideration is greater or lesser than another compound of greater or lesser viscosity (Ref. 2; D. S. Breslow, personal communication).

All of the compounds tested in Table 3 were effective as an adjuvant to BCNU chemotherapy against the LSTRA

Table 1

Timing of pyran therapy as an adjuvant to chemotherapy against the LSTRA murine leukemia

LSTRA cells (1.0×10^4) injected s.c. on Day 0. Chemotherapy consisted of BCNU, 30 mg/kg s.c., Day 7.

Days of pyran therapy (20 mg/kg/day)	Survival time (days)	Survivors[a]/ total
Untreated control	14.0 ± 0.7[b]	0/10
BCNU alone	23.4 ± 0.5	3/10
BCNU + pyran, Days 6, 7, 8	24.0 ± 1.0	3/10
BCNU + pyran, Days 6, 8, 10	27.4 ± 3.1	3/10
BCNU + pyran, Days 13, 14, 15	NS[c]	7/10
BCNU + pyran, Days 13, 15, 17	NS	8/10
BCNU + pyran, Days 20, 21, 22	NS	8/10
BCNU + pyran, Days 20, 22, 24	NS	9/10

[a] Surviving more than 90 days after tumor inoculation.
[b] Mean ± S.E.
[c] NS, not significant due to large number of long-term survivors.

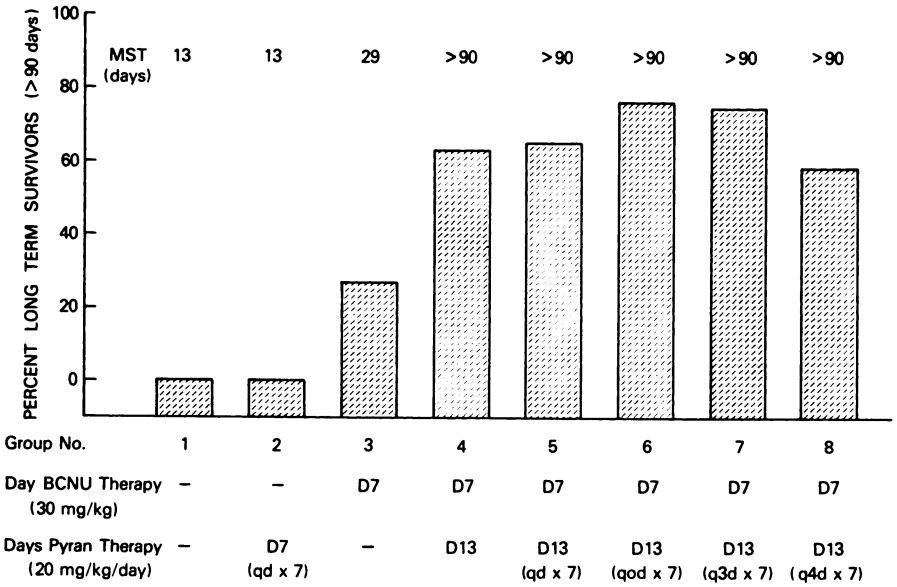

Chart 1. Effect of single *versus* multiple doses of pyran copolymer (NSC 46015) as an adjuvant to chemotherapy against the LSTRA murine leukemia. Tumor cells (1.0×10^4) were inoculated s.c. into $CD2F_1$ mice on Day (*D*) 0 and chemotherapy in Groups 3 to 8 consisted of single dose of BCNU, 30 mg/kg s.c., on Day 7. Pyran therapy was begun on Day 7 in Group 2 and on Day 13 in Groups 4 to 9 and continued at the indicated intervals to a total of 7 doses/group. Group 4 received a single dose of pyran. Median survival time (*MST*) is indicated above the *vertical bars*. Results shown are the average of 2 separate experiments each involving 12 animals/group. *Qd*, every day; *qod*, every other day; *q3d*, every 3rd day; *q4d*, every 4th day.

Table 2

Dose-response using pyran as an adjuvant to chemotherapy against the LSTRA murine leukemia

LSTRA cells (1.0×10^4) injected s.c. on Day 0. Chemotherapy consisted of BCNU, 30 mg/kg s.c., on Day 7.

Pyran dosage (mg/kg Day 13)	Survivors[a]/total	
	Experiment 1	Experiment 2
No BCNU[b]	0/10	0/10
BCNU alone	3/10	3/10
0.1	7/10	9/10
0.25		8/10
0.5		8/9[c]
1.0	7/10	6/10
2.5		6/10
5.0	9/10	7/10
10.0	9/10	10/10
25.0	8/10	8/10
50.0		9/10
100.0		8/9[c]
200.0		3/6[d]

[a] Surviving more than 90 days after tumor inoculation.
[b] Mean survival time 14.3 ± 0.5 days.
[c] One animal died from traumatic injection.
[d] Four animals died from drug toxicity without leukemia and were subtracted from the initial total of 10 animals.

Table 3

Comparison of pyran copolymer (NSC 46015) to pyrans of different viscosities as an adjuvant to chemotherapy against LSTRA murine leukemia

Chemotherapy consisted of BCNU, 30 mg/kg, Day 7 after tumor inoculation.

Pyran copolymer no.	Intrinsic viscosity $[\eta]^a$	% long-term survivors (90 days)[b]		
		1 mg/kg Day 13	10 mg/kg Day 13	5 mg/kg Days 13, 15, 17
X18720-91	0.165	50	74	70
X18571-92	0.30	67	41	70
X18720-71	0.70	59	50	78
X18720-39B	1.08	62	60	80
X18802-32	1.58	82	47	60
NSC-46015	0.76[c]	77	67	100

[a] Polyelectrolyte run as the sodium salt in 0.05 M NaCl.
[b] Should be compared to the control group (BCNU therapy alone) that produced 35% long-term survivors. Results shown are the average of 2 separate experiments each using 10 animals per group.
[c] Equivalent to about M.W. 18,000.

leukemia. However, less than 25% increase in long-term survivors over controls is probably not significant. Particularly impressive was Compound X18802-32 at low dosage and Compounds X18720-71 and X18720-39B in multiple doses. The lowest-molecular-weight pyran, X18720-91, was quite effective at the 10-mg/kg single dose or in multiple doses. None of the compounds tested were quite as effective as the original pyran, NSC 46015, if all 3 regimens are taken as a group. No signs of toxicity were observed with any of the compounds tested in the regimens used.

To determine whether this approach could be used in a different tumor system, pyran was tested against the Lewis lung carcinoma to determine its activity when used as an adjuvant to chemotherapy against a solid tumor. In Table 4, chemotherapy with 1-(2-chloroethyl)-3-(*trans*-4-methylcyclohexyl)-1-nitrosourea significantly increased the mean survival time of tumor-bearing animals by 15 days. A single dose of pyran (Group 3) 5 days after chemotherapy further increased the mean survival time by another 10 days over controls, but multiple treatments (Group 4) did not improve the response over a single dose. Therefore, the beneficial effects of adjuvant therapy with pyran copolymer are not limited to the treatment of leukemias alone.

DISCUSSION

Pyran copolymer (NSC 46015) and, to a lesser extent, its analogs of varying molecular weight have been used in several doses and time courses to treat neoplasia by adjuvant means. The observations reported are by no means conclusive due to the numbers of animals used and the number of experiments performed. However, these preliminary studies suggest that pyran adjuvant therapy may be beneficial in certain tumor systems and provide general indications concerning the use of pyran. Dosage did not appear to be critical in these studies, since no clear dose-response relationship was found (Table 2). Of significant importance, however, is that pyran appeared to be active over a large range of doses, allowing flexibility in titrating dosage to minimize toxicity while retaining good activity.

In addition, the variety of effective dosage regimens demonstrated allows some tailoring of adjuvant therapy according to the requirements of the particular tumor system. A single treatment was adequate in many experiments, although the result could usually be improved by multiple treatments. The only important restriction on dosage times was that the host must be allowed to recover from the suppressive effects of chemotherapy (3) before pyran therapy was beneficial (Table 1).

Pyran was used in a manner simulating the clinical setting in that it was used following effective chemotherapy to induce remission. Pyran alone did not alter the course of the leukemia (Chart 1, Group 2) and it has been consistently observed in this laboratory that chemotherapy is necessary for the beneficial effects of pyran to be observed. The chemotherapy lowers the tumor load in the animal and also provides the reticuloendothelial system of the host with inactivated tumor antigen. Since much of the activity of pyran appears to be mediated through the macrophage, this latter chemotherapeutic effect may be very important. The beneficial effects of pyran adjuvant therapy reported here may possibly be through timely stimulation of the macrophage to eradicate the remaining viable tumor cells after the primary tumor load has been reduced by chemotherapy. However, specific immunological testing would be necessary to confirm this hypothesis.

Lastly, it has been shown that the beneficial adjuvant activity of pyran copolymer (NSC 46015) was not limited to that particular polymer size. Significant cure rates of the leukemia were achieved using polymers of much lower and

Table 4

Pyran copolymer (NSC 46015) as an adjuvant to chemotherapy against the Lewis lung carcinoma (BDF_1 mice)

Therapeutic group	Survivors/ total	Survival time (days)	Range of death (days)
1. Lewis lung control[a]	0/12	37.0 ± 1.2[b]	32-47
2. Methyl-CCNU,[c] 20 mg/kg, Day 9 Methyl-CCNU, 10 mg/kg, Day 16	0/12	52.0 ± 3.1[d]	30-64
3. Methyl-CCNU, 20 mg/kg, Day 9 Methyl-CCNU, 10 mg/kg, Day 16 Pyran copolymer, 20 mg/kg, Day 21	0/12	62.3 ± 2.9[e]	48-76
4. Methyl-CCNU, 20 mg/kg, Day 9 Methyl-CCNU, 10 mg/kg, Day 16 Pyran copolymer, 20 mg/kg, every day, Days 21-23	0/12	60.4 ± 4.6	34-81

[a] Tumor inoculated s.c. as a 10% (w/v) homogenate in 0.3 ml Eagle's minimal essential media. Cell count approximately 1.0×10^6 cells/ml.

[b] Mean ± S.E.

[c] Methyl-CCNU, 1-(2-chloroethyl)-3-(*trans*-4-methylcyclohexyl)-1-nitrosourea.

[d] $p < 0.01$ when compared to Group 1 by t test.

[e] $p < 0.05$ when compared to Group 2 by t test.

higher molecular weights. Optimal effects were usually achieved using multiple doses except for the largest polymer (X18802-32), which was extremely effective at a single low dose. Since the toxicity of the pyran copolymers seems to increase with increasing molecular weight (2), possibly the toxic effects of NSC 46015 can be reduced without sacrificing its beneficial effects by using a smaller copolymer.

Pyran augmented the cytoreductive effect attained by initial chemotherapy of the Lewis lung carcinoma. A significantly higher increase in survival time was attained with the combined treatment than that reported for pyran treatment alone (12). Prolonged survival was accomplished when treatment was begun as late as 9 days after tumor inoculation, at a time when the Lewis lung carcinoma has metastasized (8).

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