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Experimental Antitumor Activity of Pyrazomycin[1]

Martin J. Sweeney, Fred A. Davis, Gerald E. Gutowski, Robert L. Hamill, David H. Hoffman, and Gerald A. Poore

Eli Lilly and Company, The Lilly Research Laboratories, Indianapolis, Indiana 46206

SUMMARY

Pyrazomycin (PM) completely inhibited the growth of Walker carcinosarcoma 256. Mammary carcinoma 755, Gardner lymphosarcoma, and X5563 plasma cell myeloma were inhibited more than 50% by PM. Tumors that showed more than a minimum response of 30% were the C3H and 115 mammary carcinomas, Mecca lymphosarcoma, Taper liver tumor, and Ridgeway osteogenic sarcoma. The S91 melanoma, Sarcoma 180 ascites tumors, and Ehrlich ascites solid tumor showed no significant biological responses. PM also caused several well-established dimethylbenzanthracene-induced mammary carcinomas to regress completely. The majority of the dimethylbenzanthracene-induced tumors in the treated rats regressed to some degree while most of the control tumors increased in size. The murine leukemias L1210, C1498, P1534, AKR, P388, or B82 showed no response to PM. Although PM did not inhibit the Ehrlich or Sarcoma 180 ascites tumors in mice, PM did cause up to 90% prolongation of life in rats with the Walker carcinosarcoma 256 ascites, and 9 of the 30 rats survived more than 45 days.

PM showed comparable activity against either Walker carcinosarcoma 256 or Gardner lymphosarcoma when given p.o., i.p., i.v., or i.m. It also showed comparable activity against Walker carcinosarcoma 256 when given once every 2, 3, 4, or 5 days. Plasma levels of PM-like activity were detected 3 days after a single p.o. dose of PM at 10 mg/kg in rats.

INTRODUCTION

PM,[2] a new experimental antitumor drug, is a C-riboside antibiotic (Chart 1). The compound was isolated from the fermentation broth of a strain of *Streptomyces candidus* and showed limited antifungal activity *in vitro*. PM exhibited activity against the vaccinia, herpes simplex, rhino-, and measles viruses *in vitro* and the vaccinia virus *in vivo* (6, 8). The Friend leukemia virus was also inhibited by PM as reported by DeLong *et al.* (1). Streightoff *et al.* have shown that PM may inhibit growth through its inhibition of orotidylic acid decarboxylase. PM is apparently converted to the ribosyl phosphate since PM phosphate, not PM, inhibits the decarboxylase (1, 3).

The activity of PM against the Friend leukemia virus led to the evaluation of the drug as a potential oncolytic agent. This report presents the data on PM activity against experimental tumors in mice and rats.

MATERIALS AND METHODS

Tumor Transplantation and Measurements. Solid tumor fragments were implanted s.c. by trocar in the axillary region of mice or rats. The rats and mice received single doses of PM every 3rd day except where noted. Control groups of tumor-bearing rats or mice received doses of 0.9% NaCl solution. Therapy was initiated 24 hr after implantation in the rapidly growing tumors. Treatment in the mice with the slow-growing X5563 plasma cell myeloma, Shionogi mammary carcinoma 115, and the Ridgeway osteogenic sarcoma was begun 3 to 5 days after implantation. The inhibition of tumor growth was determined by comparing the average tumor diameter ½ (length + width), of the treated group to that of the control and expressing the result as a percentage of inhibition of growth.

The DMBA-induced mammary carcinomas were treated when the tumors were 8 to 10 mm in diameter, which was 7 to 10 weeks after a single p.o. dose of 20 mg of DMBA dissolved in 1 ml of sesame seed oil. Therapy was given once every 3rd day for 18 days.

Ascites tumors of leukemias were initiated by an i.p. injection of 0.2 ml of a 1:10,000 dilution of ascites fluid or spleen homogenate from animals with an ascites tumor or leukemia. Therapy was initiated 24 hr after inoculation, and the responses to therapy were determined by comparing the average life-span of the treated group with that of the control group expressed as a percentage of increase in survival time.

Plasma Concentration of PM. The inhibition of the orotidine monophosphate decarboxylase by PM phosphate was used as the assay for plasma levels (5). Orotic acid-7-^{14}C was incubated with phosphoribosyl pyrophosphate, and 1.0 ml of plasma and the inhibition of $^{14}CO_2$ production was measured. Data are expressed as μg equivalents of PM per ml of plasma since the activated phosphorylated derivative may be present in the plasma.

[1] Presented in part at the 63rd Annual Meeting of the American Association of Cancer Research, Boston, Mass., May 1972 (7).

[2] The abbreviations used are: PM, pyrazomycin; DMBA, 7,12-dimethylbenzanthracene; Walker 256, Walker carcinosarcoma 256; aza-UdR, 6-azauridine.

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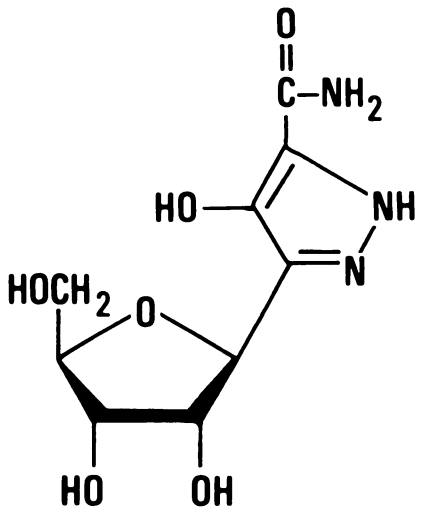

Chart 1. Structure of PM, 3-β-D-ribofuranosyl-4-hydroxypyrazole-5-carboxamide.

RESULTS

Antitumor Activity. In mice, the maximum tolerated dose of PM that can be given for 5 consecutive days is 5.0 mg/kg. At a dose of 2.5 mg/kg given for 10 consecutive days, PM showed no activity against solid tumors or leukemias in mice. DeLong *et al.* (1) reported that PM when given at 10 mg/kg every 3 days inhibited the Friend leukemia virus-induced splenomegaly. PM was tested using the every-3rd-day regimen against a spectrum of solid tumors, leukemias, and ascites tumors in mice and rats. The growth of Walker 256 was completely inhibited. The Gardner lymphosarcoma, X5563 plasma cell myeloma, and mammary carcinoma 755 were inhibited more than 50%. Five other tumors showed a biologically significant response of 30% or more and 3 showed less than 30% response (Table 1).

PM did not increase the survival time of any mice that had either the L1210, P1534, P388, or AKR lymphocytic leukemias; the C1498 myelogenous leukemia; or the Ehrlich ascites or the Sarcoma 180 ascites tumors. However, PM given i.p. or p.o. did cause a 70 and 90% increase in survival time, respectively, in rats with the Walker 256 ascites tumor. Nine of the 30 rats survived 45 days after the start of the treatment. Two of the control rats survived 45 days.

PM was given i.p. or p.o., at 10 mg/kg, to 5 rats bearing well-established DMBA-induced mammary carcinomas. The average initial tumor areas were <100/sq mm at the start of treatment. The effectiveness of therapy was assessed by the number of tumors that increased or decreased in size and the corresponding percentage of change in size, by the number of tumors that completely regressed, and by the number of new tumors that appeared during the treatment period. In the 1st test, all the tumors in the control group increased an average of 124% by the 23rd day. Three new tumors also appeared in the control group. All the tumors regressed in the group given PM i.p. There were fewer new tumors in the group treated with PM i.p. and no difference from the control group in the rats treated p.o. In the 2nd test, the majority of the tumors in the treated groups regressed. The results also indicated that the i.p. route is better than the p.o. route. There were fewer new tumors in the treated rats, although the number of new tumors in the control rats was exceptionally high (Table 2). In the 1st test, 1 rat receiving PM i.p. died on Day 18. One rat receiving PM i.p. died on Day 10, another died on Day 18, and 1 rat in control group died on Day 17. The cause of death was unknown.

Dose Response and Routes of Administration. PM is water soluble and was active against the Walker 256 and Gardner lymphosarcoma when given i.p., i.m., and i.v. PM doses of 2 to 10 mg/kg given i.p. or p.o. every 3rd day caused 70 to 100% inhibition of Walker 256. One mg/kg was the minimum dose for maximum antitumor activity. PM at 10 mg/kg i.p. caused a 53% inhibition of growth of the Gardner lymphosarcoma whereas 8 mg/kg i.p. showed minimum activity. The minimum effective dose of PM in mice is at least 8-fold greater than in rats (Table 3).

Dose Schedule. The 3-day dose schedule was used initially as a result of its effectiveness in the Friend leukemia virus. This schedule was expanded to single doses of 10 mg/kg once every 5 days which showed 100% inhibition of Walker 256. However, the same dosage given once a week was not effective. The weight gains were comparable to that of the control rats when PM was given once every 3rd day (Table 4).

Plasma Concentrations. The antitumor activity of PM when given every 3rd day indicated that plasma levels of the drug extend over a prolonged period of time. Normal Sprague-Dawley rats were given a single dose of PM, 10 mg/kg, and plasma was collected from 2 rats at each time indicated. The μg equivalents of PM were determined by the inhibition of orotidylic acid decarboxylase. The peak level of 9 to 10 μg equivalents of PM per ml occurred 6 to 8 hr after dosing either p.o. or i.m. Substantial levels were still present in the plasma: at 24 hr, 7 μg p.o. and 4 μg i.m.; at 48 hr, 3 μg p.o. and 2 μg i.m.; and at 72 hr, 1.5 μg equivalents/ml p.o. All PM activity was cleared from the plasma by 5 days (Chart 2).

Enzyme Inhibition. The inhibition of the orotidylic acid decarboxylase by PM phosphate is presumed to be the site of the inhibition of tumor growth. The resistance of the Ehrlich ascites cells *in vivo* may have to be due to lack of cell transport of PM, failure to phosphorylate PM, or the ability to resupply UMP by the uridine salvage pathway. PM was added to Ehrlich ascites and Walker 256 ascites cells *in vitro* and incubated with orotic acid-7-^{14}C and 0 to 1.7 mM PM. The $^{14}CO_2$ was collected in center wells. PM caused an 85% inhibition of the CO_2 production in the sensitive Walker 256 cells and one of 90% in the resistant Ehrlich ascites cells.

DISCUSSION

PM brings the unique pyrazole nucleus into the class of C-nucleoside antibiotics. Although the C-nucleosides have

Table 1

Summary of the activity of PM against solid tumors (10 mg/kg every 3rd day)

Tumor	Host	Tumor response to PM[a] i.p.	p.o.
Walker 256	SD rat	+++	+++
Mammary carcinoma 755	C57BL/6 mouse	++	++
Gardner lymphosarcoma	C_3H mouse	++	+
X5563 plasma cell myeloma	C_3H mouse	++	+
C_3H mammary carcinoma	C_3H mouse	+	+
Mecca lymphosarcoma	AKR mouse	+	+
Taper liver tumor	DBA/2 mouse	+	−
Mammary carcinoma 115	dds mouse	+	+
Ridgeway osteogenic sarcoma	AKR mouse	−	+
S91 melanoma	DBA/1 mouse	−	−
Sarcoma 180 (solid)	Swiss mouse	−	−
Ehrlich (solid)	Swiss mouse	−	−
DMBA-induced mammary carcinoma	SD rat	++	++[b]

[a] These data represent the range of results of 2 or more tests using 5 animals in each of the treated or control groups. The mice weighed 18 to 20 g, SD rats weighed 60 to 70 g for Walker 256, and the SD rats for the DMBA tests were 250 to 300 g at the beginning of treatment. +++, 75 to 100% inhibition of tumor growth; ++, 50 to 74%; +, 30 to 49%; −, ≦ 29%.

[b] The activity is expressed as % negative growth or regression of the DMBA-induced mammary carcinoma. Therapy was initiated on well-established tumors averaging 8 to 10 mm mean tumor diameter. Results are averages of 2 tests.

Table 2

Activity of PM against the DMBA-induced mammary carcinoma (10 mg/kg every 3rd day)

	Day 15[a]			Day 19			Day 23		
Test 1	No. of tumors increased in size	No. of tumors decreased in size	New tumors	No. of tumors increased in size	No. of tumors decreased in size	New tumors	No. of tumors increased in size	No. of tumors decreased in size	New tumors
i.p.	1 (+106)[b]	4 (−100)[4c]	1[d]	1 (+43)	3 (−100)[3]	1	1 (+14)	3 (−100)[3]	1
p.o.	4 (+82)	3 (−50)	3	1 (+256)	6 (−58)[1]	3	1 (+250)	6 (−69)[3]	3
Control	6 (+140)	0	2	6 (+177)	0	3	6 (+124)	0	3
Test 2	Day 14			Day 17			Day 20		
i.p.	2 (+81)	5 (−80)[3]	2	1 (+14)	6 (−84)[4]	2	0	5 (−93)[4]	1
p.o.	6 (+61)	1 (−100)[1]	0	3 (+77)	4 (−60)	0	1 (+188)	6 (−59)	0
Control	7 (+103)	2 (−16)	9	4 (+95)	4 (−21)	10	6 (+52)	3 (−36)	11

[a] Treatment was begun when the average areas were < 100 sq mm and was continued once every 3 days for 21 days and a total of 8 doses in Experiment 1 and 7 doses in Experiment 2. The last measurement in Experiment 1 was 2 days after the last dose.

[b] No. in parentheses, % increase or decrease in size.

[c] Superscript designates the number of complete remissions.

[d] Indicates the number of new tumors that appeared during the treatment period.

some antibacterial and antifungal activities, few have antitumor activity (2).

Although PM showed some effect on 10 of 13 tumors studied, the regression of the DMBA tumor by PM was the most noteworthy. The DMBA tumor is a slow-growing primary tumor. It is similar in some respects to some human breast cancer inasmuch as it is also prolactin dependent in the early period of growth. We have used several of the standard clinical drugs against the DMBA tumor with no effect.

The inhibition of the orotidine 5'-monophosphate decarboxylase of resistant Ehrlich cells *in vitro* indicates that PM does cross the cell membrane and that it is phosphorylated in both sensitive and resistant cells. Perhaps the uridine salvage pathway is sufficient in resistant cells to resupply the UMP requirements. Streightoff *et al.* (6) reported that PM had no antiviral activity in the presence of uridine.

Most drugs are excreted within 24 hr; therefore, the prolonged plasma level of PM is unusual. This may explain the ability of PM to exert its biological activities even when dose is given every 3 or 4 days. No data are available yet on the tissue distribution of PM. We have detected PM and 1 minor metabolite in the urines of mice, rats, and rabbits. The metabolite has not been identified but apparently possesses a free enolic 4-hydroxyl group, as determined by a positive $FeCl_3$ test.

PM inhibits the decarboxylation of orotidine monophosphate, as does aza-UdR. However, these 2 compounds differ in 2 respects. First, the C—C ribose-pyrazole linkage in PM is stable to both chemical and enzymatic hydrolysis, whereas the C—N ribose-pyrimidine linkage in

Table 3
Effectiveness of PM given by various routes of administration

Tumor	Dose (mg/kg) every 3rd day × total doses	i.p.		p.o.		i.m.		i.v.	
		% I[a]	AWC (g)	% I	AWC (g)	% I	AWC (g)	% I	AWC (g)
Walker 256	10 × 5	100	−5.4	100	+11.0	100	−5.2	82	+15.4
	8 × 5	91	+5.2	100	+25.6	100	−7.0		
	6 × 5	100	+38.6	86	+9.6	100	+5.4		
	4 × 5	81	+30.6	62	+40.4	100	+14.6		
	0		+36.6		+11.2		+29.2		
Walker 256	6 × 5	100	+7.8	100	+3.2				
	4 × 5	100	+12.0	93	+16.0				
	3 × 5	100	+11.0	93	+17.4				
	2 × 5	71	+21.6	70	+16.0				
	1 × 5	81	+22.0	37	+19.6				
	0		+34.4		+38.0				
Gardner lymphosarcoma	10 × 5	53	−1.4	41	−1.6	51	−2.8	50	−4.2
	8 × 5	46	−3.2	22	−0.6				
	6 × 5	23	+0.6	22	−0.6				
	4 × 5	32	+0.6	7	+3.4				
	0		+4.4		+4.0		−4.0		+3.3

[a] %I, % inhibition of tumor growth; AWC, average weight change in rats or mice.

Table 4
Activity of PM given at various dose schedules against Walker 256 (10 mg/kg i.p.)

Dose schedule	Total[a] doses	Av. wt change (g)	% inhibition of tumor growth
Daily	3	−25	No survivors
Every 2nd day	5	+4	100
Every 3rd day	4	+37	100
Every 4th day	3	+26	100
Every 5th day	2	+45	100
Every 7th day	2	+51	0
Control (0.9% NaCl solution)	10	+35	

[a] The 1st dose is given 24 hr after tumor implantation. Animals were dosed for 10 days and the tumors were measured on the 11th day.

aza-UdR is subject to both chemical and enzyme hydrolysis. When aza-UdR is given p.o., it is hydrolyzed to 6-azauracil, a suspected cause of central nervous system disturbances in man (4). Second, in animals, PM is active against murine tumors when given once every 3 to 5 days, whereas aza-UdR requires multiple daily doses (4).

These differences in the properties of PM compared with aza-UdR are sufficient to warrant its consideration for clinical evaluation in the treatment of cancer in man. Acute and subacute toxicology studies in animals are in progress.

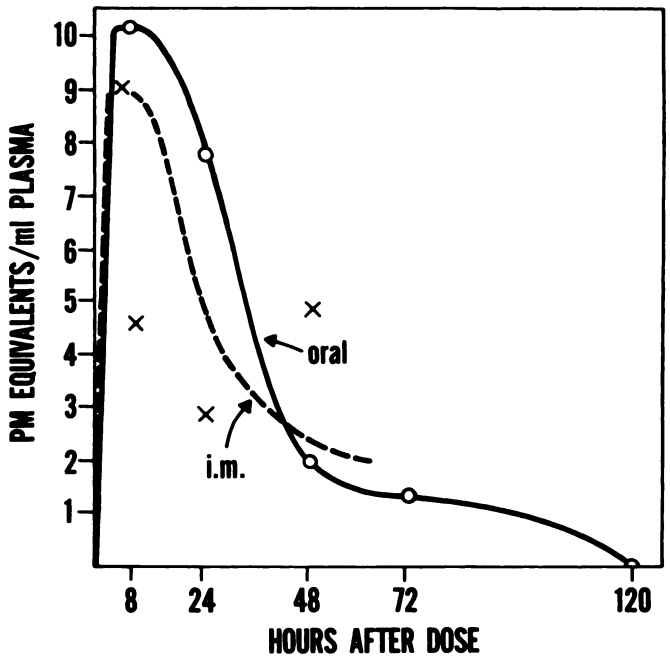

Chart 2. Plasma concentrations of PM. PM at 10 mg/kg was given to rats. Two rats were bled by heart puncture at each time period. One ml of plasma was assayed for PM-like activity as described in "Materials and Methods."

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