Attenuation of Certain Neoplasias by Chlorphenesin (36631)

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Chlorphenesin (3-*p*-chlorophenoxy-1,2-propanediol) has been shown by several techniques to suppress humoral antibody response (1–3) without inducing tolerance or affecting antigenic priming of immunocompetent cells (4). The compound is essentially nontoxic at therapeutically active levels, does not alter carbon clearance by the RE system, and does not reduce protection by preexisting antibody (5). It has also been found to inhibit the release of both histamine and SRS-A from primate cells (6, 7). Because of its unique characteristics as a noncytotoxic immune modifier, studies have been undertaken to evaluate the effects of chlorphenesin on several virus-induced murine leukemias, and to assess its action against a variety of transplanted neoplasms. This report describes our recent experimental findings, and summarizes preliminary results from clinical trials of the drug.

Materials and Methods. Rauscher murine leukemia virus (RMLV) was derived from stock provided by Dr. F. J. Rauscher of the National Cancer Institute, and has been maintained for a number of years in our laboratory. Friend murine leukemia virus (FMLV) was obtained from the American Type Culture Collection. Both viruses have been maintained by periodic intraperitoneal (ip) passage in female Balb/c mice. Uniform reference pools were prepared from clarified homogenates of infected spleen tissue and were stored at −70° until used. In the studies described, virus samples were rapidly thawed and diluted in Hanks' balanced salt solution (HBSS) to provide $10^{-1.5}$ tissue equivalent doses/ml (1 TED = 1 g spleen tissue/ml). Young randombred female Swiss mice (Taconic Farms) were inoculated ip with 0.2 ml of virus suspension and randomly distributed into paired groups of 18–20 mice each. This dose of virus routinely produced 80% mortality in leukemic control groups within 50–60 days.

Leukemia L-1210 was obtained from Dr. Michael Chirigos of the National Cancer Institute, and has been carried in the ascitic form by ip passage in B6DF1 Jax mice. In the present studies, ascites cells were harvested, diluted in HBSS, and implanted subcutaneously in B6DF1 mice.

Rauscher ascites leukemia (RAL) is a highly malignant lymphoblastic line originally derived from a lymphoma occurring in a Balb/c mouse infected with RMLV. This tumor was provided by Dr. O. J. Plescia of Rutgers University, and has been maintained by weekly ip passage in Balb/c mice. In test procedures, RAL cells were implanted intradermally, giving rise to discrete and progressively growing malignant tumors.

Additional studies in which chlorphenesin was evaluated against a wide spectrum of transplantable tumors have been carried out at the Sloan-Kettering Institute using methods which have been described previously (8, 9).

In the experiments reported here, chlorphenesin was administered twice daily in divided doses, since the drug has a half-life in rodents of about 4 hr (10). The compound was dissolved in warm HBSS to provide an ip dose of 100 mg/kg in 0.5 ml, which was administered in the morning and late afternoon of each treatment day. Because of its limited aqueous solubility, when greater concentrations were required the compound was suspended in sterile 0.2% methyl cellulose (Methocel, 100 cps) in saline. In all experi-

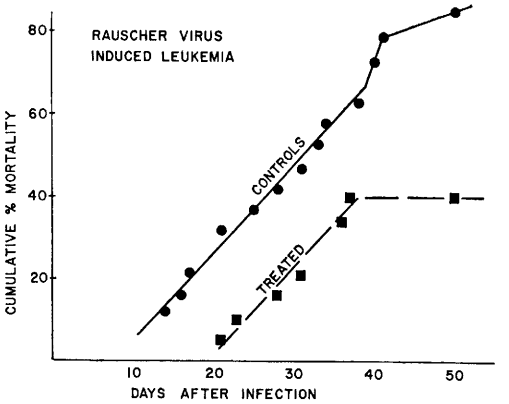

FIG. 1. Rauscher virus-induced leukemia. Female Swiss mice infected ip with RMLV on day 0; 20 mice/group. Treated: chlorphenesin administered ip at 2 × 100 mg/kg on days 1, 2, 3, 4, 7, and 8. Controls: treated ip on same schedule with diluent only (HBSS).

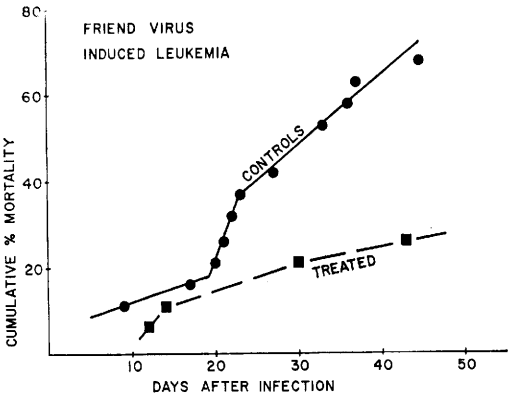

FIG. 2. Friend virus-induced leukemia. Female Swiss mice infected ip with FMLV on day 0; 19 mice/group. Treated: chlorphenesin administered ip at 2 × 100 mg/kg on days 1, 2, 6, 7, 9, 12, and 13. Controls: treated ip on same schedule with diluent only (HBSS).

ments, control animals received injections of diluent or vehicle using the same schedule and volume as for drug-treated groups.

Results. Chlorphenesin therapy was found to exert a pronounced sparing effect on leukemic mortality after infection with Rauscher virus. Results of a typical experiment are shown in Fig. 1. Delayed onset of early death in drug-treated animals was observed in this case, but the most characteristic finding was the marked sparing effect in later stages of the disease. As shown in Fig. 1, mortality in treated animals leveled off at 40% while controls continued to die at a nearly linear rate. Note that drug treatment in this experiment was completed within 8 days after virus infection, more than 4 weeks before the mortality plateau became apparent.

Similar therapeutic effects were obtained against Friend virus-induced leukemia, as shown in Fig. 2. Chlorphenesin was again administered twice daily, but in this case using a pulsed regimen continued through day 13.

Since both of the above systems represented virus-induced neoplasms, the possibility that chlorphenesin might be acting through an antiviral effect was considered and evaluated. The drug had no detectable effect upon *in vivo* infections with influenza A, vaccinia, Columbia SK, or murine hepatitis viruses, nor did it reduce the biological activity of several viruses (NDV, influenza, Friend virus) reacted directly with the compound *in vitro* prior to bioassay. Chlorphenesin had no interferon inducing activity in mice, and neither enhanced nor reduced *in vivo* response to interferon induction by endotoxin, xerosin, or Newcastle disease virus.

These results suggested that chlorphenesin was probably acting upon malignant cells rather than against the transforming virus *per se*, and studies were initiated to test the compound against a variety of transplantable tumors. Leukemia L-1210 in ascites form was used for initial experiments, and it was quickly found that chlorphenesin had little effect against conventional massive ip doses

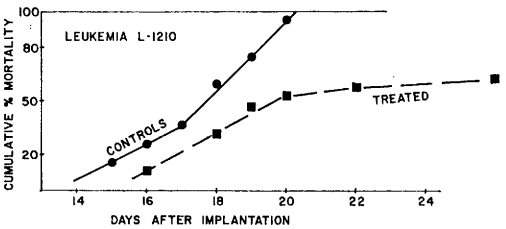

FIG. 3. Leukemia L-1210. Female BDF1 (Jax) mice implanted sc with 100 L-1210 cells/mouse on day 0; 19 mice/group. Treated: chlorphenesin administered ip at 2 × 100 mg/kg on days 1, 4, 5, 6, and 8. Controls: treated ip on same schedule with diluent only (HBSS).

TABLE I. Effect of Chlorphenesin Therapy on RAL Tumors in Balb/c Mice.[a]

Parameter	Day	Controls (12)	Chlorphenesin treated (13)
% Tumor incidence	12	92	39
	14	100	54
	19	100	45
Mean tumor diameter (mm)	12	4.8	1.8
	14	7.2	2.1
	19	11.1	3.4
Cumulative % mortality	19	33	15
	24	58	38
	30	90	45

[a] Mice: female Balb/c, 14–16 g at time of implant. Implant: 200 RAL cells/mouse, intradermally. Treatment regimen: chlorphenesin ip, 2 × 100 mg/kg/day on days 3, 5, 7, 10.

of this highly malignant line. However, when the system was modified by using reduced numbers of cells implanted subcutaneously, the sparing effect was readily demonstrable, as shown in Fig. 3. In this experiment more than 40% of the treated mice survived until the experiment was terminated at 50 days, at which time they had no visible evidence of residual tumor.

Similar results were obtained against intradermal implants of RAL cells in syngeneic (Balb/c) hosts. Using this system, an implant of 200 cells gave rise to a localized and progressively growing lethal tumor which could be readily visualized and measured directly with calipers. Data from such an experiment are summarized in Table I. Animals treated with chlorphenesin showed decreased incidence of tumors, reduced mean tumor size, and enhanced survival compared to controls.

Further studies were carried out at the Sloan-Kettering Institute against a wide spectrum of transplantable tumors. Of 11 screening systems used, chlorphenesin has been found to be inactive in 7, while showing posi-

TABLE II. Transplantable Tumors Resistant to Chlorphenesin Therapy.

Tumor designation	Chlorphenesin dosage tested (mg/kg/day)	Parameter of activity	Day	Response treated/control (T/C ratio)
LPC-1 myeloma	25–400	60 day survival	60	0/0
Melanoma B-16	50–100	Tumor diam	8	0.89–0.98
			15	0.91–0.97
Carcinoma C-1025	100	Tumor diam	13	0.90
			20	0.81
Meeca lymphosarcoma	100	Tumor diam	8	1.04
		Surv. time		15.4/15.6
Ridgeway osteogenic sarcoma	50–400	Tumor diam	13	0.72–0.80
			20	0.77–0.95
Taper liver tumor (ascites)	100	Av total packed cell vol	7	1.22
Taper liver tumor (solid)	400	Tumor diam	7	1.07
			14	1.02

TABLE III. Transplantable Tumors Responsive to Chlorphenesin Therapy.

Tumor designation	Chlorphenesin dosage (mg/kg/day)	Treatment regimen	Parameter of activity	Day		Response T/C
Carcinoma EO-771	2 × 50	ip Days 1–6	Tumor diam	8		0.58
				15		0.18
	2 × 100			8		0.56
				15		0.22
Walker carcinoma 256	2 × 100	ip Days 1–6	Tumor diam	8		0.42
				15		0.45
Ehrlich ascites carcinoma	2 × 50	ip Days 1–6	Av total packed cell vol	7		1.04
	2 × 200					0.29
Sarcoma 180	2 × 25	ip Days 1–6	Tumor diam	8		0.86
	2 × 100					0.80
	2 × 200					0.47–0.78
	2 × 100	ip Days 1, 2, 4, 5, 6	Long-term tumor regression		Rx	Controls
				15	0/10	0/10
				22	1/10	1/10
				29	3/10	1/10
	Single experiment, confirmatory studies underway			43	5/10	1/10
				50	4/10	1/10
				60	4/10	1/10
			% Mortality	60	40	80

tive effects against 4 tumor lines. Table II summarizes results from those tumors against which chlorphenesin has shown no activity to date at the dosage levels indicated. Table III presents pertinent data on those tumor systems which have responded to chlorphenesin therapy, including Carcinoma EO-771, Walker carcinoma 256, Erlich ascites carcinoma, and Sarcoma 180. Of particular interest are the suggestions of reduced mortality and enhanced regression rate in Sarcoma 180. Confirmatory studies of this aspect are underway.

In sharp contrast to the toxic symptoms produced by therapy with drugs such as cyclophosphamide or amethopterin, mice treated with therapeutically active levels of chlorphenesin showed no evidence of systemic cytotoxicity. In addition, studies with normal mouse L-cells in tissue culture failed to demonstrate cytotoxic effect at drug concentrations below 1000 μg/ml (1000 mg/kg). Further experiments involved freshly harvested L-1210 or RAL cells which were incubated *in vitro* with graded concentrations of chlorphenesin. Cell suspensions were then diluted and bioassayed for tumorigenic potential in susceptible hosts. Data from three such studies are presented in Table IV, and confirm the absence of cytotoxic effect at concentrations of less than 1000 μg/ml, which is at least 20 times greater than blood levels obtainable *in vivo* at active dosage levels. These results strongly suggest that antitumor effects of chlorphenesin are not based on cytotoxic action, although the possibility of such action by a metabolic end product has not been completely excluded.

Based on the findings described above, preliminary clinical trials in cancer patients were undertaken by the Clinical Screening Group of the European Organization for Research on Treatment of Cancer (Dr. G. Mathé). To date, 31 patients have been treated for periods ranging from 1 to 6 weeks. The oral dose of chlorphenesin was from 1 to 6 g daily, with the usual dose being 4 g/day. These patients had a wide range of neoplasms, all

TABLE IV. Biological Activity of Tumor Cells After Reaction *in Vitro* with Chlorphenesin.

Reaction conc (μg/ml of chlorphenesin)	No. of cells implanted/ mouse (ip)	No. of mice/ group	Mean time to 50% mortality (days)
Leukemia L-1210 (10^5 cells/ml, 37° for 2 hr)			
0	200 cells	23	13.3
100		24	13.0
200		24	13.2
400		23	13.4
800		23	13.7
Leukemia L-1210 (10^5 cells/ml, 37° for 1 hr)			
0	1000 cells	18	11.2
1000		18	11.9
Rauscher ascites leukemia (RAL) (10^5 cells/ml, 37° for 1 hr)			
0	500 cells	21	13.8
500		21	13.1
1000		21	12.9
3000		22	—[a]

[a] Mortality did not exceed 5% in this group. All reactions were carried out in diluent of Hanks' BSS containing 5% calf serum. Reactions at zero concentration were in diluent only.

established by biopsy diagnosis. Chlorphenesin treatment was ineffective in 16 cases of carcinoma of the cervix, uterus, tonsil, esophagus, and lung, and in 4 cases of sarcoma. However, in 9 cases of squamous cell carcinoma of the skin, including one penile carcinoma, apparently complete remission was achieved in one (Dr. J Hewitt, Paris), and substantial though incomplete regression was observed in 4 others (Drs. J. Hewitt, J. M. Spitalier, J. F. Paullet, Marseille). No benefit was observed in two patients with basal cell skin carcinoma.

Discussion. Chlorphenesin has been found to have therapeutic activity in a wide variety of experimental neoplasias, yet the mode of action remains undefined. Although drug treatment enhanced survival in several virus-induced murine leukemias, available evidence seems to exclude antiviral action as a basis for the effect. Similarly, studies on transplantable tumors indicate significant activity, yet cytotoxic action by the compound is absent at therapeutically active levels.

Taking these studies as a whole, several patterns begin to emerge. First, chlorphenesin shows no apparent activity in experimental neoplastic systems utilizing massive input doses resulting in overwhelming disease and rapid death. Conversely, the drug has been active in situations where malignancy progresses more slowly and which allow sufficient time for host response to come into play. Secondly, the observed sparing effect generally becomes manifest well after discontinuance of drug therapy. This is most apparent in the Rauscher virus and Sarcoma 180 studies, but can also be seen from the results with Carcinoma EO-771. These factors suggest to us that chlorphenesin may act through host immune mechanisms directed against malignant cells.

In light of this compound's known ability to suppress humoral antibody response (4) and to block passive cutaneous anaphylaxis (5), one possible mode of action might involve interference with synthesis or binding of enhancing antibody to tumor cells, thus permitting more efficient recognition of tumor antigens by host immune mechanisms. At present, we postulate that chlorphenesin acts by augmenting cell-mediated immunity. Although definitive proof is not yet available, several observations tend to support this hypothesis: (a) Chlorphenesin does not suppress T-cell priming or secondary response (4); (b) the drug does not delay and sometimes accelerates skin allograft rejection in dogs (Dr. D. Blumenstock, personal communication); (c) optimal effects against experimental tumors are delayed in onset and seem to occur only in situations most opportune for development of host resistance. Furthermore, it has recently been shown that chlorphenesin may markedly elevate levels of cyclic AMP in treated cells (7), an event known to be associated with activation of immunocompetent lymphocytes (11) and directly involved with inhibition of tumor cell growth *in vitro* (12).

Most drugs currently used for cancer chemotherapy are broadly cytotoxic and highly immunosuppressive, thus creating major problems of management in already debilitated patients. Although early clinical results

with chlorphenesin demonstrate only qualified promise, it is hoped that these studies will help pave the way for future development of nontoxic compounds that may enhance host immune response against growth of malignant cells.

Summary. Chlorphenesin (3-*p*-chlorophenoxy-1,2-propanediol) has shown antineoplastic activity in a number of experimental tumor systems, including virus-induced murine leukemias and several transplantable tumors. Therapeutic activity was most evident in neoplasias characterized by a protracted course, and optimal drug effects were manifested well after discontinuance of treatment. The drug was not cytotoxic at therapeutic levels, and had no detectable antiviral activity. Experimental results suggest that chlorphenesin may act by enhancing cell-mediated immune responses of the host. Preliminary clinical trials indicate that chlorphenesin may be of value in the treatment of squamous cell carcinoma of the skin.

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