Intracerebral Growth and Treatment of Leukemia L1210. (25936)

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Our preliminary studies of disposition of leukemia cells (L1210) in different tissues of DBA/2J and CDBA† mice, implanted subcutaneously with the tumor, have shown that the brain becomes infiltrated 1-3 days prior to death.‡ It was interesting to determine whether intracerebral (I.C.) route of inoculation of lymphoid leukemia (L1210) would lend itself to studies such as: 1) Time required for disease to become systemic. 2) Degree of leukemic infiltration in different brain areas. 3) Effect of drug therapy when there is high concentration of leukemic cells in the brain, when ability of the drug to penetrate blood-brain barrier could be of considerable importance. Data are presented showing that intracerebrally injected leukemia (L1210) grows in the brain, infiltrates diffusely in the arachnoid, and rapidly becomes systemic. Significant increases in survival time were obtained with drug therapy.

Materials and methods. 10-12-week-old DBA and CDBA male mice§ weighing 22 to 28 g were employed. For each experiment a DBA mouse bearing subcutaneous (S.C.) implant of leukemia (L1210) in the right inguinal region was sacrificed by cervical dislocation on 9th day after inoculation.‖ A midline incision was made and the diaphragm exposed and transversely cut. Blood was collected by cardiac puncture into heparinized syringe. One volume of whole blood was diluted with 9 volumes of sterile saline (0.9% NaCl). DBA or CDBA mice were injected intracerebrally by penetrating the skull with 25 gauge needle in area of midbrain and slowly introducing 0.05 ml of inoculum, using a microliter syringe (Hamilton Co.). In comparative studies separate groups of mice were inoculated S.C. in the right inguinal region with the same volume of inoculum. Brain and spleen tissue, removed from either I.C. or S.C. injected mice, was prepared as a mash. The tissue was inserted into 1 or 2 ml syringe and forced through 20 gauge needle into equivalent volume of sterile saline. The mash was passed back and forth through the needle into syringe barrel until uniform tissue suspension was obtained. Whole blood was collected by cardiac puncture as previously described. Sterile saline was used for all dilutions.

Results. Table I shows that 3 days after inoculation of leukemic blood into the brain, tumor cells were in sufficient concentration that S.C. inoculation of 0.1 ml of 2% brain inoculum resulted in tumor growth and death in recipient mice. Leukemic titer in brain increased so that by fifth and eighth day after injection of leukemic cells into the brain, dilutions of 0.4 to 0.02% of brain suspension were capable of eliciting tumor growth and subsequent death.

Comparison was made of time required for the disease to become systemic, when I.C. and S.C. routes of inoculation of leukemia (L1210) were employed (Table II). Two groups of CDBA¶ mice were implanted, I.C. or S.C., with 0.05 ml of a 10% concentration of leukemic whole blood, prepared as previously described. On days indicated following inoculation, I.C. and S.C. implanted mice were sacrificed; whole blood, brain, and spleen tissue were taken and used for subcutaneous inoculation into CDBA mice.

Results from Table II show that spleen was sufficiently infiltrated to transmit the disease at 2 days following I.C. injection, and that leukemic cells were present in circulating blood by the sixth day. In contrast, by the

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† (BALB/cAn x DBA/2J)F_1 hybrid male.

‡ Chirigos, M. A. (unpublished data).

§ Obtained from NIH breeding colony.

‖ Median survival time (MST) for these mice is usually 10-13 days.

¶ CDBA male mice are susceptible to growth of leukemia (L1210), Goldin, *et. al.*(1).

TABLE I. Potency of Inoculum Prepared from Brain Tissue of Mice Inoculated Intracerebrally* with Leukemic Blood (L1210).†

	Day intracer. inoculated mice were sacrificed for brain tissue inoculum					
	Day 3		Day 5		Day 8	
% conc. of inoculum‡	Dead/Total	Range of death (days)	Dead/Total	Range of death (days)	Dead/Total	Range of death (days)
50	3/3	12-14	3/3	12–13	3/3	14
10	"	"	"	13	"	15–16
2	"	13–16	"	15–17	"	16–19
.4	0/3		2/3	16–18	"	17–20
.08	"		1/3	18	"	17–22
.02	"		0/3		1/3	20

* Median survival time of intracer. inj. mice was 9 days.
† DBA/2J mice were used throughout experiment.
‡ All inocula diluted with sterile saline and inj. subcut., 0.1 ml/mouse, in right inguinal region.

sixth day, leukemia was not transplanted successfully with blood, spleen, or brain taken from mice inoculated by the S.C. route; however, by eighth day they were capable of transmitting the disease.

Pathology. Sections of central nervous system were examined in DBA mice sacrificed on eighth day following intracerebral injection of leukemia (L1210). Diffuse infiltration by leukemic cells in the subarachnoid spaces was noted (Fig. 1). Small nodules of leukemic cells were seen in the ventricles, and occasional intracerebral blood vessels were surrounded by a cuff of leukemic cells.

Amethopterin and 3′,5′-dichloroamethopterin have been shown to prolong the lifespan of mice with advanced leukemia (L1210)(1) when the S.C. route of tumor inoculation was employed. Although leukemic cells are present in the brain late in the course of the disease, following S.C. inoculation, they are apparently not in as high concentration as when inoculated I.C.

It was of interest to determine whether treatment with these drugs could increase survival time of mice inoculated I.C. with leukemia (L1210). Results of a representative experiment are summarized in Table III. Drug therapy was begun on seventh day after mice were injected I.C. with leukemic blood, since biological evaluation of brain tissue from I.C. injected mice and degree of infiltration in brain as observed microscopically indicated that the brain is well infiltrated by this time. In this experiment, since all controls succumbed, the higher inoculum was at least 10 times greater than that required to give 100% deaths from tumor. Although there were no

TABLE II. Disposition of Leukemia in Tissues of Mice Inoculated Intracerebrally or Subcutaneously with Leukemia (L1210).*

	Day tissue inoculum was prepared from mice													
Inoculum†	Day 2		Day 4		Day 6		Day 8		Day 10		Day 11		Day 12	
	D/T‡	R‡	D/T	R	D/T	R	D/T	R	D/T	R	D/T	R	D/T	R
	Intracerebral mice§													
Blood	0/3		0/3		3/3	14–15	3/3	12	1/1	9	No intracer. survivors on days 11 and 12			
Spleen	2/3	14–16	3/3	14–16	"	10–11	"	8–9	"	7				
Brain	"	14–16	"	11–12	"	9–12	"	8–10	"	9				
	Subcutaneous mice‖													
Blood	0/3		0/3		0/3		3/3	14	3/3	10–14	3/3	11–12	2/2	9
Spleen	"		"		"		"	10–12	"	8	"	8	"	7
Brain	"		"		"		"	14–17	"	12–13	"	12	"	11

* CDBA mice were used throughout this experiment.
† 0.1 ml of undiluted whole blood, .2 ml of a 50% concentration of spleen or brain implanted subcut. in right inguinal region.
‡ D/T = Dead/Total. R = Range of death in days.
§ Median survival time, 10 days.
‖ " " " , 13 " .

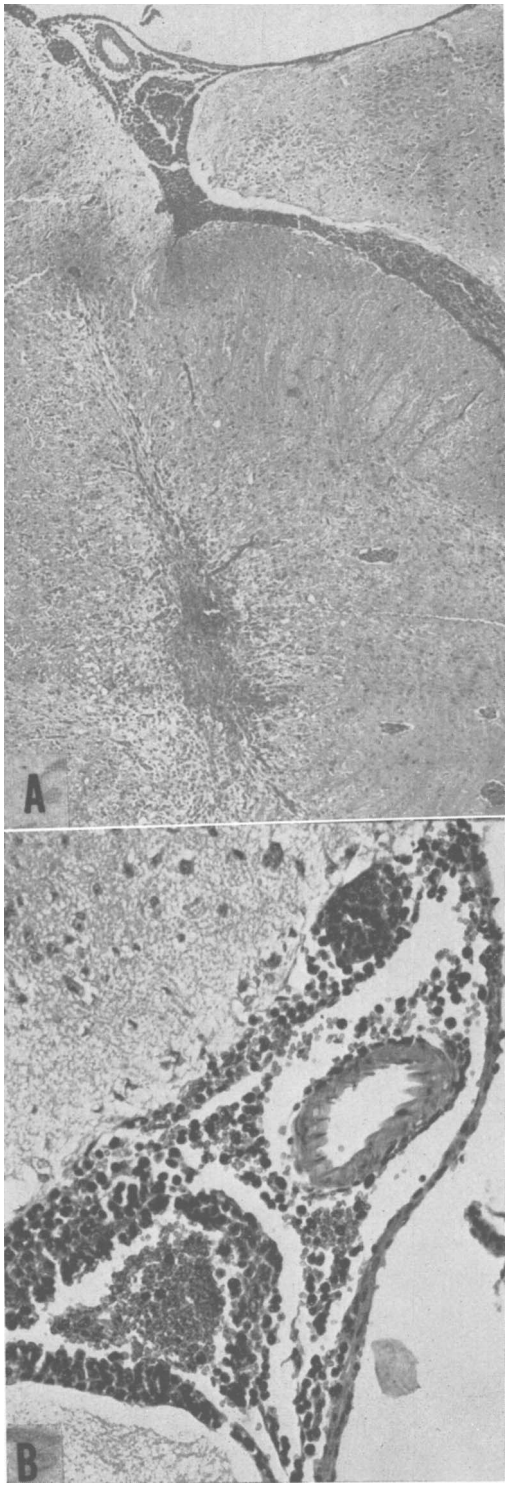

FIG. 1. (A) Photomicrograph of midbrain of mouse inj. intracer. with leukemia L1210. Needle puncture tract in midbrain is hemorrhagic and surrounded by inflammatory cells. No leukemic cells noted along needle tract. There is diffuse leukemic cell infiltration in arachnoid and a few intracerebral vessels exhibit perivascular cuffing. 40 ×.

FIG. 1. (B) Leukemic cells fill arachnoid spaces on surface of midbrain and infiltrate wall of vein. 178 ×. Stain, hematoxylin-eosin.

TABLE III. Effect of Drug Therapy on Mice Implanted Intracerebrally with Leukemic Blood (L1210).*

Conc. of blood inoculum (%)†	Drug treatment‡	Median survival time (days)	Range of death (days)
10	DCM	41	15–68
"	MTX	19	15–30
"	None	12	11–12
1	DCM	52	14–66
"	MTX	28	23–29
"	None	12	12–13

* DBA/2J mice; 10/group.

† All inocula diluted with sterile saline, and inj. intracer., .05 ml/mouse.

‡ DCM = 50 mg/kg 3′, 5′-dichloroamethopterin.
MTX = .75 mg/kg amethopterin.

Drug therapy was initiated on 7th day after mice were implanted intracer., and administered subcut., daily, in axillary region.

survivors in the treated groups, the results show that the antifolic agents caused substantial increases in survival time with both levels of inoculum.

Whether earlier treatment of I.C. injected mice would have led to complete regression of leukemia in brain has not been determined.

That both drugs were effective indicates that these drugs may be capable of penetrating the blood-brain barrier. Studies are presently being conducted with this system to determine penetrability of these and other agents known to be effective against leukemia (L1210).

Summary. Mouse leukemia (L1210) was grown successfully when introduced by intracerebral route. Brain tissue was rapidly infiltrated and the disease became systemic 2-3 days following I.C. inoculation. Deaths occurred somewhat sooner than when the disease was inoculated S.C. Administration of antifolic agents (amethopterin and 3′,5′-dichloroamethopterin) increased survival time.

1. Goldin, A., Humphreys, S. R., *J. Nat. Cancer Inst.*, 1960, v24, 283.

Received May 23, 1960. P.S.E.B.M., 1960, v104.