Asparaginase Clearance: Influence of the LDH-Elevating Virus[1] (34402)

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The therapeutic effects of L-asparaginase upon certain leukemias and other malignancies have been convincingly documented (1–14). It is noteworthy that the curative effects observed in mice have been dramatic; whereas, the responses in patients have been significant but, unfortunately, not comparable to the rapid and prolonged disappearance of established, disseminated leukemia observed with mice (15–17). These therapeutic differences stimulate further examination of the possible influencing factors involved.

The basic mechanism of the therapeutic action of asparaginase appears on the surface to be clear (18–25). Some leukemia and tumor cells require an exterior source of asparagine since there appears to be a metabolic defect which prevents these malignant cells from synthesizing their own asparagine (26). Such cancer cells are thus dependent upon the blood supply of asparagine carried as a part of the circulating endogenous amino acid pool. Administration of an appropriate dose of the enzyme asparaginase reduces the level of such required blood-borne asparagine to some minimal "therapeutic" concentration. This treatment may be considered a form of indirect chemotherapy whereby the cancer cells are destroyed by being deprived of a nutritionally required substrate.

Materials and Methods. Mice employed for determining asparaginase clearance rates and for testing for the presence of the LDH-virus in tumor-bearing animals were Swiss ICR female weanlings, 18–22 g, and known to be free of the LDH-virus and certain other interfering agents (27–29). The experimental mice were inoculated intraperitoneally 55 days prior to asparaginase administration with 0.1 ml of infected mouse plasma having an LDH-virus titer of $10^{10.5}$ median infectious doses (ID_{50})/ml.

Commercially prepared L-asparaginase (Worthington), having 20 IU/mg, was employed to prepare an enzyme solution containing 375 IU/ml. This was injected intraperitoneally in 0.2-ml volumes to give 75 IU/mouse. The blood concentration of asparaginase was tested periodically. This dose and route of administration is similar to that employed in earlier therapeutic studies (1, 3, 9, 10).

To determine the relative rates of asparaginase disappearance from the peripheral blood, periodic samples were collected from each mouse by the orbital bleeding procedure (30). The plasma was separated in the bleeding tubes by centrifugation and its asparaginase concentration was determined by standard methods (31).

Plasma infectivity tests (PIT) were employed to confirm the presence of the LDH-virus in mice that exhibited an elevated plasma LDH since an increase in enzyme activity is not in itself proof of LDH-virus infection. Suspected plasmas were inoculated intraperitoneally into mice with predetermined normal plasma LDH levels; after 48–72 hr the recipient mouse plasmas were again tested. A five to tenfold increase in LDH was considered presumptive proof of LDH-virus transmission.

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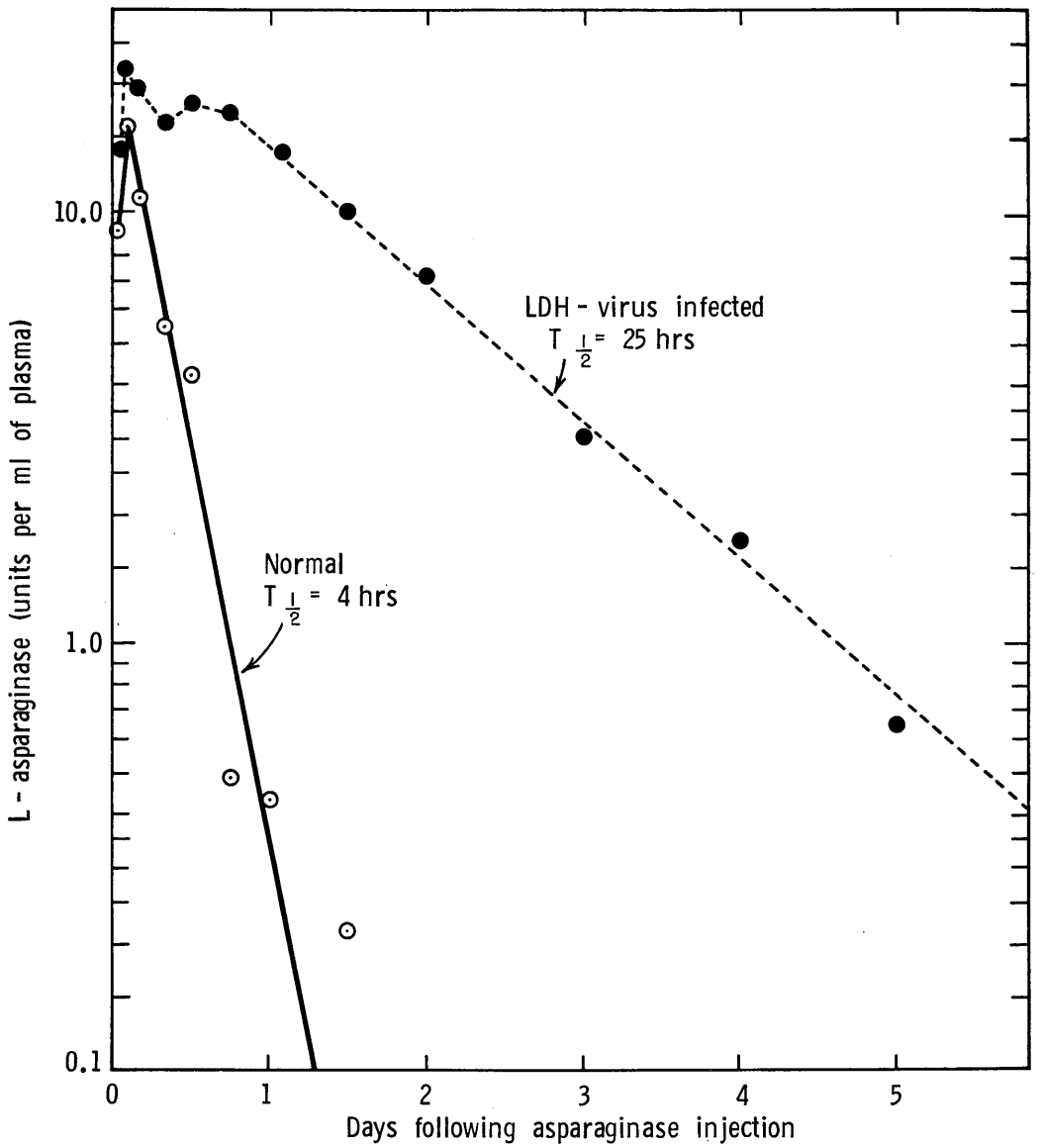

FIG. 1. Disappearance rate of L-asparaginase from plasma following intraperitoneal injection: comparison of clearance in normal mice with that of mice chronically infected with the LDH-elevating virus.

Lactate dehydrogenase (LDH) activity was determined by a semimicro modification of the Wroblewski-LaDue procedure utilizing an automatic recording spectrophotometer to measure the rate of change of optical density at 340 mμ as a function of the LDH conversion of $NADH_2$ to NAD, while the added substrate pyruvate was oxidized to lactate. For details, see Ref. (33).

Results. Figure 1 demonstrates the differential rates of disappearance of an exogenous dose of EC-2 L-asparaginase from the blood of normal mice compared with those chronically infected with the LDH-virus (32–36). The rates shown are equivalent to an enzyme half-life ($T_{1/2}$) of about 4 hr for normal mice and 25 hr for LDH-virus-infected animals. Experimental results were similar for

TABLE I. Assay of Normal and Tumor-Bearing Mice for Plasma LDH Levels and for the Presence or Absence of the LDH-Elevating Virus.

Normal or tumor-bearing mice	Mouse strain	Plasma LDH values	LDH-virus (PIT)[a]
Normal mice	BAF_1[b]	300	(—)
	BAF_1	400	(—)
	BAF_1	300	(—)
EARAD-1	BAF_1	4000	(+)
	BAF_1	95,000	(+)
	BAF_1	23,400	(+)
EARAD-1 (resistant)	BAF_1	229,000	(+)
EL-4	C57BL/6	19,900	(+)
ERLD	C57BL/6	58,400	(+)
K-36	BKF_1[b]	28,700	(+)
RAD A1	A	6700	(+)
ASL1 (from bank)[c]	A	66,000	(+)
	A	70,000	(+)
EARAD-1 (tissue culture)	BAF_1	37,200	(—)
	BAF_1	11,000	(—)
Gardner 6C3HED (33)	C3H	Elevated	(+)
P1534 Leukemia (33)	DBA/2	Elevated	(+)
P1798 Leukemia (33)	Balb/C	Elevated	(+)

[a] PIT: Plasma infectivity test for LDH-virus (33).
[b] $BAF_1 = (C57BL/6 \times A)F_1$; $BKF_1 = (C57BL/6 \times AKR)F_1$.
[c] Tumor bank storage at —70° for 18 months.

both acutely infected mice which had carried the virus for 18 hr and for chronically infected mice which had been inoculated 55 days prior to asparaginase administration. The minimum time factor required, however, for inducing host clearance impairment is of importance in understanding some of the paradoxical observations concerning seeming differences in asparaginase action against newly implanted tumors treated 1 hr following implantation, compared with the surprisingly better therapeutic effects against established 7-day-old tumors. The virus must be present in the host for about 12 hr before it induces the clearance impairment illustrated in Fig. 1.

Table I demonstrates the results obtained when an assortment of standard transplantable mouse tumors commonly used by investigators for asparaginase research were assayed for the presence or absence of the LDH-elevating virus. It may be noted that all tumors tested carried the virus with the exception of one line of EARAD-1 which had been carried routinely in cell culture. The majority of experimental asparaginase studies have been carried out with the Gardner 6C3HED lymphoma and the X-radiation-induced EARAD-1 originating in a Sloan-Kettering laboratory (2).

Discussion. The data illustrated in Fig. 1 bear primarily upon the relative maintenance of the asparaginase level in the blood of the host and indicate the potential therapeutic benefits of inducing a benign impairment in the host clearance processes during drug administration. For example, if the original asparaginase dose in the virus-free mice were 10 times that given the virus-infected animals, within 24 hr the asparaginase concentration in the blood of the virus-free mice would nevertheless have dropped to a level below that found at 24 hr in the virus-infected mice. As an alternative, if repeated doses of asparaginase were given to the virus-free mice every 4 hr in an effort to simulate the drug level of the delayed clearance curve observed in the virus-infected mice, it would

require a total amount of asparaginase 6 times the single dose given the virus-infected mice in order to maintain a similar drug concentration for 48 hr (41).

Since it now seems clear that the LDH-virus was unknowingly present with the transplanted leukemia cells of other investigators, as demonstrated in Table I, these experiments offer an explanation for the paradoxical observations of Mashburn *et al.* (7–9), and of Boyse *et al.* (1), to the effect that asparaginase appeared to be more effective when administered against established 7-day leukemias than when the same amount was administered to mouse recipients 1 hr after leukemia or tumor implantation.

In the latter case, most of the administered asparaginase would have been eliminated by the host prior to the induction of an impaired clearance by the virus, which would be initiated only after about 12 hr (40, 41).

These direct measurements of comparative asparaginase clearance, in the presence and absence of the virus (40), provide evidence for the astute speculations and inferences concerning the possibility of clearance modification by LDH-virus which have been raised by Broom (37) and Old *et al.* (38).

Therapy experiments, employing asparaginase in the presence and absence of the LDH-virus, demonstrated even more directly the importance of the virus in obtaining apparent cures (39). In the case of LDH-virus-free mice with established, disseminated leukemia, no survivals were observed in animals receiving single doses of 100 IU of L-asparaginase. This was in contrast to 95% survival at 40 days of similarly treated mice which had been intentionally infected with authentic lactate dehydrogenase-elevating virus.

No evidence presently exists for a direct influence of the LDH-elevating virus upon tumor regression in this system. In most instances tumor growth rate in the virus-infected hosts was comparable to that of the tumor control groups. Thus the virus-associated elevation of plasma LDH has no direct bearing upon the observed enhancement of asparaginase therapy. The plasma LDH elevation, however, is related to the virus-induced mechanism which is responsible for the slowed removal of administered asparaginase. This, in turn, is involved in the improved asparaginase therapy (40, 41).

Summary. Asparaginase, injected in conventional therapeutic doses into mice previously inoculated with the lactate dehydrogenase-elevating virus (LDH-virus), disappeared from the peripheral blood at a significantly slower rate than in corresponding virus-free mice. Direct testing of the transplanted tumors and leukemias employed in the earlier studies on the antitumor properties of asparaginase have indicated that the virus, was unknowingly present. The evidence indicates that the virus, through its impairment of host enzyme clearance and possibly other factors, played a significant role in the observed therapeutic effects of EC-2 L-asparaginase against sensitive mouse leukemia and lymphosarcoma.

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