L-Asparaginase for the Treatment of Cancer

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Introduction

The amino acid, L-asparagine, is a nutritional requirement of both normal and cancer cells. Unlike normal cells, however, certain leukemic cells cannot synthesize asparagine and must consequently rely on an external supply in the plasma and tissues. The administration of the enzyme, L-asparaginase, destroys this free source of asparagine, starving and killing certain cancer cells.

In 1953, Kidd¹ noted that guinea pig serum had antitumor activity against two strains of murine lymphoma and a strain of lymphosarcoma in rats. It was subsequently shown by Broome² in 1963 that the tumor inhibitory activity of the serum was due to L-asparaginase, which is present in high concentrations in the sera of guinea pigs and other members of similar species. In 1966, DeLowrey³ and his associates using a purified guinea pig serum treated a boy with acute lymphoblastic leukemia, and obtained an objective response. However, it was not until the isolation of an L-asparaginase from Escherichia coli, with similar antitumor activities, that sufficient quantities of L-asparaginase could be synthesized for large-scale clinical trials.

In 1967, Hill⁴ and his associates, and Oettgen,⁵ first described the induction of complete remissions. Hill’s paper discussed three patients treated with L-asparaginase prepared from E. coli. These three patients had acute lymphoblastic leukemia. One of these patients achieved a complete remission after 32 days of therapy; however, four weeks after discontinuation of L-asparaginase, the patient relapsed. Oettgen reported therapeutic response in four patients with acute lymphoblastic leukemia and one with acute myeloblastic leukemia; however, he had no response in two further patients with acute myeloblastic leukemia and two with lymphosarcoma.

Even with the use of the L-asparaginase derived from E. coli, supplies were initially limited and each batch of new material required extensive testing to determine not only its activity but also its potential toxicity. At this time, there was considerable concern regarding its possible bacterial product contamination and many of the initial side effects, especially nausea and vomiting, were attributed to contamination of the original preparations. After repeated exposure to L-asparaginase, some patients developed sensitization and serious if not fatal anaphylactic reactions could occur. It was thought that this sensitization was possibly due to impurities in the initial preparations and further purification has, in fact, modified this risk of sensitization.

Although the initial hopes that L-asparaginase would occupy a major and unique role in the treatment of acute leukemia have not been forthcoming, it is still an effective agent in the treatment of some cancers, particularly childhood acute lymphoblastic leukemia. However, with the use of L-asparaginase in large doses over a short period of time, in combination with other known effective chemotherapeutic agents, it has been possible to prolong the survival of
children and also adults with acute leukemia. The high-dose, short-term use of L-asparaginase has successfully averted the risk of sensitization and anaphylactic reactions. However, many of the other reversible side effects have still been seen following even the administration of the more purified preparation.

The Pharmacology of L-Asparaginase

L-asparaginase, intravenously injected into patients, is primarily distributed in the plasma. The concentration of the enzyme in the plasma is dose-related and a significant level can be detected in plasma for as long as 23 days after cessation of the enzyme treatment. The plasma half-life of L-asparaginase varies from eight to 30 hours in different patients and is not affected by sex, age, surface area, diagnosis, extent of disease or hepatic and renal functions of patients. The half-life is not altered by daily treatment. Repetitive treatment results in a cumulative increase in the plasma level of L-asparaginase. This provides the basis for a less than daily administration of the enzyme. In fact, the weekly administration of the enzyme has been shown to have indistinguishable therapeutic effects from the daily treatment. This would also minimize the possible development of clinical resistance.

L-asparaginase therapy has evoked allergic reactions, but the incidence has not been as great as anticipated. After obtaining a complete remission with L-asparaginase, some patients were maintained on L-asparaginase for several months and showed no signs of anaphylactic reactions. However, development of immunological reactions in some cases is associated with the rapid clearance of plasma L-asparaginase. The decline in enzyme activity generally precedes the anaphylactic reaction by two to four days, and on the day the reaction occurs there is virtually no enzyme activity detectable in the plasma. The occurrence of anaphylactic reactions in man is somewhat dependent upon the preparation of the enzyme used. Preliminary reports, however, suggest that it is a general phenomenon and the plasma of sensitized patients exhibits a positive precipitin reaction to the enzyme. It is also possible that before the L-asparaginase therapy, patients are immunosuppressed. This could be associated with the cancer or a result of prior treatment with immunosuppressive anticancer agents. In addition, it has been shown that L-asparaginase itself inhibits the immune response.

Riley's "lactic dehydrogenase elevating virus" is frequently associated with experimental tumors and markedly prolongs the plasma L-asparaginase halftime in mice. This indicates that the virus, through its impairment of the rate of enzyme clearance in hosts, plays an important role in the therapeutic effects of L-asparaginase against leukemia and lymphosarcoma in rodents. Therefore, plasma lactic dehydrogenase levels and L-asparaginase half-times were determined in 15 patients who had been treated with L-asparaginase. No difference in the plasma L-asparaginase half-life was observed between patients with elevated and normal lactic dehydrogenase levels.

L-asparaginase is a protein with a molecular weight of 139,000. The apparent volume of distribution of L-asparagin-
ase in man exceeds the plasma volume by 20 to 30 percent. Thus, 70 to 80 percent of the administered enzyme appears to remain in the plasma. Lymph samples collected from a thoracic duct fistula of a patient being treated with L-asparaginase showed that the movement of the enzyme from the vascular to the extravascular extracellular space (lymph) was slow and incomplete. At three hours, a maximum concentration of L-asparaginase approximately 20 percent that of plasma was achieved. The poor transport of L-asparaginase from vascular to extravascular extracellular space limits its usefulness against solid tumors.

The concentration of L-asparaginase in the cerebrospinal fluid was less than one percent that of plasma level, and only traces were found in the urine. Studies have demonstrated that substances with molecular weights exceeding 90,000 will not pass readily out of the capillaries. The kidney glomerulus in man will not allow particles with molecular weights over 46,000 to pass. It is therefore not surprising that L-asparaginase (Mol. wt. 139,000) was found only in traces in the cerebrospinal fluid and urine.

It has been demonstrated that after the injection of L-asparaginase into the mouse, the enzyme activity persisted in the liver after its clearance from the blood and other organs. This suggested that L-asparaginase may be excreted by the hepatobiliary system and/or taken up or phagocytized by the reticuloendothelial system. In dogs, less than five percent of the enzyme was excreted in bile during a 24-hour period, and thus the bile excretion could not account for the major enzyme loss from the plasma. Zymosan, an inhibitor of the reticuloendothelial system, did not alter the clearance rate of L-asparaginase in either dogs or guinea pigs, suggesting that the reticuloendothelial system was probably not involved with the clearance of the enzyme. However, to date, the possible involvement of the reticuloendothelial system in clearing the enzyme has not been studied in man.

It is assumed that the primary action of L-asparaginase is the elimination of circulating pools of L-asparagine resulting in an increase of ammonia and L-aspartic acid. The L-asparagine level was reduced in leukemic cells of four patients following L-asparaginase treatment. The level of L-asparagine was highest in the cells of one patient who responded to therapy, but was unmeasurable within four days of the first dose of the therapy. L-asparaginase therapy depresses plasma L-asparagine to nearly undetectable levels for three weeks. An L-asparagine "rescue" infusion was given to patients with the acute brain dysfunctional syndrome. The high concentration of ammonia in the blood (700-900 µg percent) resulting from the L-asparaginase treatment has been shown not to generally impair the function of the central nervous system (CNS). However, in view of the fact that the function of the CNS could be affected by decreased amounts of L-glutamine or by increased concentrations of L-aspartic acid and L-glutamic acid, it is rather interesting that improvements were seen in some of the patients after L-asparaginase "rescue" infusion. In addition, the exogenous L-asparaginase rescue remedy could not be effective immediately following the L-asparaginase treatment, since the infused L-asparagine may be hydrolyzed by large
amounts of circulating L-asparaginase.

**Mechanisms of Resistance**

The major limitation of L-asparaginase treatment in acute leukemia is the rapid development of clinical resistance. Accelerated clearance of L-asparaginase by immunological mechanism has been reported in patients developing resistance. A second mechanism might involve the development of more efficient ways of utilizing L-asparagine, either from the plasma or from cells in which it is not totally depleted by the L-asparaginase treatment or from which L-asparagine was actively synthesized by asparagin synthetase. In human leukemic cells, the resistance is at least in part related to the asparagin synthetase activity. Before L-asparaginase therapy, asparagine synthetase is undetectable in human leukemic cells; after therapy the cells from L-asparaginase resistant patients showed a sevenfold increase in asparagine synthetase.

**Toxicity of L-Asparaginase**

Almost all patients treated with L-asparaginase experienced symptoms of anorexia and nausea. Not infrequently, these symptoms can be severe and associated with mild to moderate vomiting, and loss of more than 5 percent of the body weight was seen in 50 percent of the reported series of patients. Occasionally, sensitivity is seen with initial exposure to drugs. This sensitivity reaction occurs immediately after the administration of the drug and is associated with fever, dyspnea, hypotension, agitation, and epigastric pain. Although these symptoms do not necessarily preclude the continuation of therapy, careful subsequent administration of the drug is advisable. Skin testing with low doses of L-asparaginase for hypersensitivity before and after therapy have failed to demonstrate any hypersensitivity reactions, even in those patients who showed an increased sensitivity to the intravenous drug.

Mild to moderate alterations in liver function studies, particularly associated with decreasing fibrinogen, albumin and cholesterol levels are seen. The rapidity of the depression in the plasma fibrinogen levels does not correlate with the dose of L-asparaginase and in some patients the fibrinogen levels returned to normal with the continuation of therapy. In the remainder, recovery was seen within seven to 10 days after discontinuation of the L-asparaginase. A marked drop in serum cholesterol levels was also detected in a large proportion of cases, with levels as low as 80 mg./100 mls. being recorded. In more than 50 percent of patients reported in one series, there was a drop of more than 1 gm./100 mls. of albumin during the administration of L-asparaginase. The decrease in plasma fibrinogen levels during the administration of L-asparaginase was slow and suggested a reduction in the synthesis rate, rather than increased utilization. These clinical findings were substantiated using autologous labeled fibrinogen in patients receiving L-asparaginase where normal half-life of this radioactive fibrinogen was demonstrated. In a further study, however, increased fibrin-split products were seen in a few patients receiving L-asparaginase; despite this increase, only a few patients developed frank hemorrhage. Alterations in other liver function tests were associated with the intravenous administration of L-asparaginase, but
these regularly reverted to normal after cessation of therapy. These changes included elevation of serum, transaminases, alkaline phosphatase, and bilirubin in about 50 percent of cases. Similar alterations in these liver function tests were seen with the administration of other cancer chemotherapeutic agents. However, in an autopsy series, more than 50 percent of those patients who were receiving the drug at the time of death showed evidence of moderate to severe fatty infiltration of the liver. These changes appear to be reversible after completion of drug therapy, as patients dying two months following L-asparaginase administration showed no evidence of fatty infiltration.

Nonketotic hyperglycemia occurred in seven of 49 patients treated with L-asparaginase alone. This appeared to be dose-related and did not occur at levels below 250 international units/M²/day. The hyperglycemia appeared to be related to a decrease in the serum immuno-reactive insulin; this could possibly be due to decreased insulin levels, destruction of the insulin molecule by L-asparaginase, decreased insulin secretion, or interference with insulin synthesis. Small doses of exogenous insulin, however, could readily control this nonketotic hyperglycemia and it appears most likely that L-asparaginase interfered with the synthesis of the insulin molecule by blocking the incorporation of L-asparagine into the human insulin molecule. In autopsy material of patients with hyperglycemia associated with L-asparaginase administration, it was clearly shown that it was marked hypertrophy of the islet cells, again suggesting a compensating hypertrophy, in an attempt to synthesize insulin in the presence of L-asparaginase.

Small areas of local pancreatitis have also been noted in patients during the administration of L-asparaginase; however, these areas appear insufficient to cause low levels of circulating insulin and generally, although there was evidence of increasing urinary amylase, the serum amylase was not elevated during the periods of severe hyperglycemia.

Electroencephalograms performed on patients receiving L-asparaginase almost uniformly showed reduced activity and diffuse slowing. A smaller number of patients experienced marked lethargy associated with mental confusion. There have been occasional reports of hallucinations, confused states and development of inappropriate behavior. Although meningeal leukemia was present in a number of these patients, the remainder were free of any anatomical explanation and showed improvement of the electroencephalographic changes following the completion of L-asparaginase administration. These electroencephalographic changes were probably due to metabolic causes and the most likely explanation appeared to be the elevation of the blood ammonia; when these levels returned to normal, which they did rapidly after completion of therapy, the electroencephalographic changes also improved to normal.

The majority of side effects occurring with the administration of L-asparaginase appeared to be the result of its effect on protein synthesis; the depression in protein synthesis appears to rapidly revert to normal following the completion of L-asparaginase administration. The L-asparaginases produced by different organisms are not cross reacting. If allergic phenomena are experienced with
The Immunosuppressive Activities of L-Asparaginase

The antitumor activity of L-asparaginase, particularly against lymphoid tumors, both in experimental animals and in man, led to the investigation of L-asparaginase and its related compound, L-glutaminase, as a possible immunosuppressive agent. The initial in vitro studies showed that very small doses as low as one international unit/ml. inhibited lymphocyte blastogenic responses to phytohemagglutinin (PHA) almost completely, and using morphological criteria and the incorporation of $^3$H-thymidine, $^3$H-muridine, or $^{14}$C-leucine, there was evidence of inhibition or morphological transformation of the lymphocytes, which was associated with inhibition of deoxyribonucleic acid and ribonucleic acid and protein synthesis. It was also noted that the lymphocytes from patients receiving L-asparaginase therapy for neoplastic disorders responded poorly to PHA. It was thought that this was the result of circulating L-asparaginase, particularly as the patients' serum would inhibit blastogenic responses of normal subjects, and normal serum restored the responses of the patients' lymphocytes. The inhibition of the PHA response in vitro was used to assess L-asparaginase survival times in serum. After a single intravenous dose of 82,000 international units/M$^2$ inhibitory concentrations of L-asparaginase were present in the patients' serum from 10-18 days following infusion. The finding that L-asparaginase was immunosuppressive and not myelosuppressive has encouraged further work in the investigation of the specific enzyme the patient produces as an approach to immunosuppression to prevent organ allograft rejection.

Clinical Results with L-Asparaginase

After the initial encouraging results using purified extract of guinea pig serum, and the isolation of L-asparaginase from E. coli, commercial production of L-asparaginase was started using various sources. The majority of L-asparaginase viable for clinical trial was prepared from the organism E. coli, but supplies have also been prepared with varying degrees of purity from other organisms such as Serratia marcescens and Erwinia carotovorum, as well as the purified supply from agouti (a species similar to the guinea pig) serum.

Initial clinical trials with L-asparaginase were performed in patients who had become refractory to conventional chemotherapeutic regimens. As with all new drugs, the initial dose was small, and there was a gradual escalation of the doses used until drug toxicity was apparent. Although the drug was used to treat acute lymphoblastic leukemia, acute granulocytic leukemia, chronic lymphocytic leukemia, chronic granulocytic leukemia, various forms of lymphoma and solid tumors, the major effect was in those tumors of lymphoid origin. Patients with acute lymphoblastic leukemia, either adults or children, achieved complete remission, whereas the patients with nonlymphoid malignancies including acute myeloblastic leukemia appeared to respond less well and a small number achieved only short-term, complete or partial remissions. The patients who received L-asparaginase for the treatment of acute lymphoblastic
leukemia achieved complete remissions with doses as low as 400 international units/M², doses up to 20,000 international units/M²/day x 28, and although there appeared to be no definite relationship between the dose received and the number of patients achieving complete remission, there appears to be a better response rate in those patients receiving 10,000 units/M²/day x 28. In this group of patients the median duration of unmaintained remission was considerably greater than those receiving 8,000 international units/M² for 28 days.

Although the initial results in the treatment of acute lymphoblastic leukemia with L-asparaginase were extremely encouraging, the rapid development of resistance (see pharmacology) and the risk of anaphylaxis associated with repeated administration prevented it from becoming more universally used as a single agent for this disease.

During the initial clinical trials with L-asparaginase as a single agent in the treatment of refractory leukemia, continued animal studies are being performed in particular to determine whether L-asparaginase would be additive or synergistic when used with other drugs known to be effective in the control of acute leukemia. In mouse leukemia studies, particularly with leukemia L51786, L-asparaginase was shown to be additive when given with Vincristine or with Daunomycin. In the more sensitive system, Leukemia ERADI, prior therapy with cytosine arabinoside and thioguanine followed by L-asparaginase therapy, produced a significant increase in the number of 50-day cures in those animals treated with subsequent L-asparaginase. These preliminary animal studies have paved the way to extensive use of L-asparaginase in combination with other chemotherapeutic agents known to be effective in the control of human cancers.

L-asparaginase has been used in combination with many other chemotherapeutic agents known to be effective in the treatment of acute leukemia L-asparaginase, in combination with other agents, particularly cytosine arabinoside and Daunomycin, has been used in acute leukemia and these combinations appear especially favorable because of the lack of myelosuppression. With L-asparaginase, both drugs have been able to be used in full therapeutic doses. Although the majority of responses with the combination regimens appears to be in those patients with acute lymphoblastic leukemia, responses have been reported with combination chemotherapy in patients with acute myelogenous leukemia. In the majority of protocols using L-asparaginase in combination, however, this drug is used for one course of therapy only, primarily because of its toxic side effects and the rapid development of resistance.

L-asparaginase does not diffuse across the blood brain barrier because of its high molecular weight. It is, however, a useful agent in the therapy of meningeal leukemia, depleting the supply of L-asparagine essential in the metabolism of intrathecal leukemia cells.

**Conclusion**

The unique properties of L-asparaginase, including the ability to block incorporation of the nonessential amino acid L-asparagine into cancer cells, has made this agent a particularly exciting drug.

It is possible that depletion of L-aspar-
aggregate from L-asparagine-dependent cells of patients could be enhanced by hemodialysis. Preliminary studies have shown that hemodialysis removed a large amount of L-asparagine, but homeostatic mechanisms prevented a decrease of L-asparagine in the plasma. Dietary deprivation of L-asparagine is another approach; L-asparagine is in the amino acid constituent of vegetables, fruits and most proteins. Since the deprivation of folate acid from the diet was not as successful as one would have hoped, L-asparagine deprivation may not be practical. L-asparagine analogs, 5-diazo-4-oxo-L-norvaline and 5-chloro-4-oxo-L-norvaline are effective inhibitors of asparagine synthetase, and the analogs of cofactors of asparagine synthetase might also be useful. L-glutamine is required for the biosynthesis of L-asparagine, and the analogs of glutamine, such as azaserine and 6-diazo-5-oxo-L-norleucine, were found to be the inhibitor of asparagine synthetase and were synergistic against tumors in experimental systems. The clinical trial of a combination of L-asparaginase and azaserine has been reported, however, further studies are required to prove that combination therapy is significantly superior to L-asparaginase used alone.

The rapid development of resistance and its toxic side effects have limited use to single courses of L-asparaginase alone or in combination with other agents. The investigation of other enzymes with similar characteristics but with less toxic side effects may prove useful for the control of cancer.

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