

Delayed-type Hypersensitivity in Response to L-asparaginase in a Case of Acute Lymphoblastic Leukemia

Hidehiko Narazaki, Kiyohiko Kaizu, Chiharu Miyatake,
Shinya Koizumi, Takeshi Asano and Osamu Fujino

Department of Pediatrics, Nippon Medical School Chiba Hokusoh Hospital

Abstract

L-asparaginase (L-Asp) is an important reagent for acute lymphoblastic leukemia because asparagine is required for the malignant growth of tumor cells, especially lymphoblastic leukemia cells. An allergic response to L-Asp is not unusual because L-Asp is derived from *Escherichia coli* and is often recognized as a foreign protein. The hypersensitivity induced by L-Asp is of the immediate type in most cases. We report on a 5-year-old girl who was hospitalized for precursor T-cell lymphoblastic leukemia. She was treated according to a Tokyo Children's Cancer Study Group protocol (TCCSG ALL L09-1603 HEX/BFM). During the intensification phase, blisters with erythema developed on the arm proximal to the catheter insertion site owing to a delayed-type hypersensitivity reaction caused by intravenous L-Asp administration. She was treated with additional methylprednisolone, tapered dexamethasone, and an antihistamine for the allergic reaction. No asparaginases other than *E. coli* L-Asp have been approved for use in Japan. Other asparaginases, such as polyethylene glycol L-Asp and *Erwinia* L-Asp should be quickly approved for use as alternative chemotherapy reagents in Japan.

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Key words: asparaginase, delayed-type hypersensitivity, lymphoblastic leukemia

Introduction

Asparagine is an amino acid required by cells for the production of proteins. Tumor cells, especially lymphatic tumor cells, require huge amounts of asparagine to fuel their rapid, malignant growth and use both dietary asparagine and self-synthesized asparagine to satisfy their needs. In the 1960s, L-asparaginase (L-Asp) isolated from *Escherichia coli* was found to inhibit the growth of lymphoblastic

leukemia cells¹ by exploiting their unusually high requirement for asparagine. Furthermore, L-Asp enzymatically decomposes asparagine into aspartic acid and ammonia. Therefore, L-Asp is an important chemotherapy agent for acute childhood lymphoblastic leukemia².

However, many adverse effects of L-Asp have been documented. The major adverse effects include liver dysfunction, blood clotting disorders, diarrhea, vomiting, pancreatitis, low blood levels of antithrombin III or fibrinogen, and mild bone

Correspondence to Hidehiko Narazaki, Department of Pediatrics, Nippon Medical School Chiba Hokusoh Hospital, 1715 Kamagari, Inzai, Chiba 270-1694, Japan
E-mail: hide.narazaki@gmail.com
Journal Website (<http://www.nms.ac.jp/jnms/>)

marrow suppression. Anaphylaxis is also an important adverse effect. In addition, hypersensitivity with repetitive L-Asp use can occur in 6% to 43% of cases³. Because L-Asp is derived from *E. coli*, it is recognized as a foreign protein by the patient's immune system.

Several chemotherapy agents might be used instead of *E. coli* L-Asp. Asparaginase derived from *Erwinia chrysanthemi* is expected to have the same effect as *E. coli* L-Asp and has been approved for the treatment of acute lymphoblastic leukemia in patients with hypersensitivity to *E. coli* L-Asp⁴. A complex of polyethylene glycol (PEG) conjugated with L-Asp (is also used to treat acute lymphoblastic leukemia⁵. This agent is PEGylated to reduce potential immunogenicity while preserving its activity and prolonging its half-life. However, these agents have not been approved for use in Japan.

Case Description

A 5-year-old girl visited our hospital with facial edema, swollen cervical lymph nodes, and dyspnea. A chest X-ray film revealed a swollen thymus, severe pleural effusion in the right lung, and hepatosplenomegaly. She had no history of allergy. Initial laboratory examinations (**Table 1**) revealed a white blood cell count of 73,450/ μ L with 81% blast cells among the peripheral leukocytes. The expression of cyCD3, CD5, CD7, CD34, CD99, and CD244 were confirmed on blast cells with flow cytometry; therefore, precursor T-cell lymphoblastic leukemia was diagnosed. Gene abnormality, for example, that of SIL-TAL1, was not detected on chimeric gene screening. The patient was admitted to our hospital, and treatment was started according to a Tokyo Children's Cancer Study Group protocol

Table 1 Initial laboratory findings

Initial laboratory findings						
WBC	73,450 / μ L	CRP	1.4 mg/dL	PT	1.27 INR	
Stab	1.0 %	ESR	21 mm/hr	APTT	33 sec	
Seg	10.0 %			Fib	117 mg/dL	
Lym	5.0 %	ALT	12 U/L	ATIII	86 %	
Mono	2.0 %	AST	28 U/L	FDP	6.9 mg/mL	
Eosino	1.0 %	LDH	927 U/L	D-D	4 mg/mL	
Baso	0.0 %	T-Bil	0.6 mg/dL	TT	1.29 INR	
Blast	81.0 %	UA	6.8 mg/dL			
RBC	3.94×10^6 / μ L	BUN	17 mg/dL	sIL-2R	1,548 U/mL	
Hb	11.0 g/dL	Cre	0.5 mg/dL	Ferritin	20 mg/L	
Plt	0.128×10^6 / μ L	TP	6.7 g/dL	Urine β 2MG	53.3 ng/mL	
Ret	0.7 %	Alb	4.1 g/dL			
Bone marrow tap			Flow Cytometry of peripheral blood (%)			
NCC	301,430 / μ L (Blast 95%)		cyCD3	100	CD34	77.8
PO positive cell	3 %		CD7	99	CD99	94.9
			CD5	92	CD56	0.5
			CD8	21	CD244	98.0
			HLA-DR	0		

WBC = white blood cell, Stab = stab cell, Seg = segmented cell, Lym = lymphocyte, Mono = monocyte, Eosino = eosinophil, Baso = basophil, RBC = red blood cell, Hb = hemoglobin, Plt = platelet, Ret = reticulocyte, CRP = C reactive protein, ESR = erythrocyte sedimentation rate, ALT = L-aspartate: 2-oxoglutarate aminotransferase, AST = L-alanine: 2-oxoglutarate aminotransferase, LDH = lactate dehydrogenase, T-Bil = total bilirubin, UA = uric acid, BUN = blood urea nitrogen, Cre = creatinine, TP = total protein, Alb = albumin, PT = prothrombin time, APTT = activated partial thromboplastin time, Fib = fibrinogen, ATIII = antithrombin III, FDP = fibrin/fibrinogen degradation products, D-D = D dimer, TT = thrombotest, s-IL-2R = soluble interleukin 2 receptor, β 2MG = β 2 microglobulin, NCC = nucleated cell concentration, PO = peroxidase, CD = cluster of differentiation

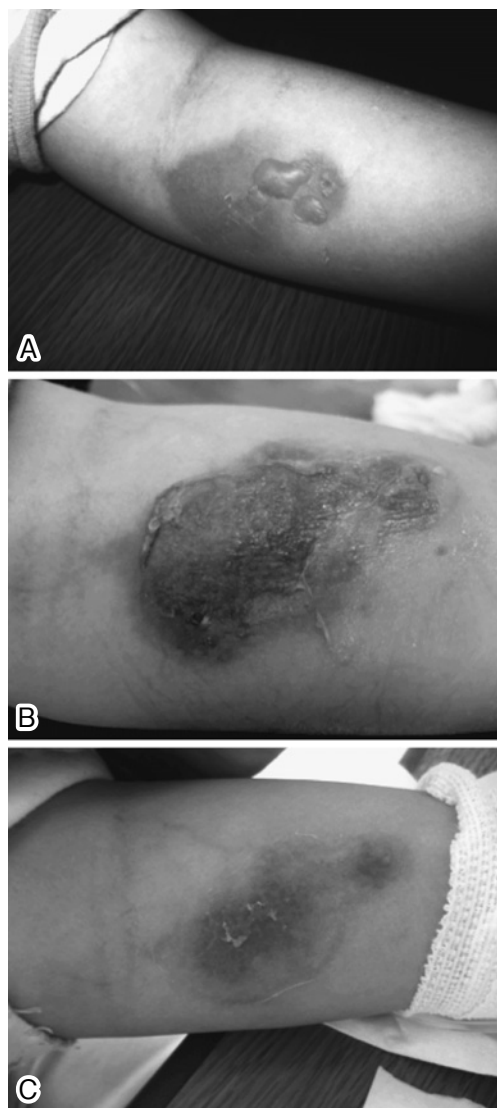


Fig. 1 A. Left forearm of the patient after 5 days of L-Asp administration. Blisters and erythema are observed proximal to the catheter insertion site.
 B. Left forearm of the patient after 9 days of L-Asp administration. The blister has ruptured, and erosion is observed.
 C. Left forearm of the patient after 2 weeks of L-Asp administration.

(TCCSG ALL L09-1603 HEX/BFM). This protocol specifies that L-Asp should be intravenously administered a total of 19 times, including 9 times in the induction phase. Subsequently, there are 6 intensification phases, wherein dexamethasone, L-Asp, and other several chemotherapy reagents are administered. Dexamethasone is administered on days 1 to 5, and L-Asp is administered on day 6. Dexamethasone administration is tapered from day

Table 2 Measurement of anti L-Asp antibodies

	Anti-L-Asparaginase	
	IgE	IgG (U/mL)
After 2nd intensification phase	3	1
After 3rd intensification phase	3	3

*IgE antibody titers to control normal human serum, expressed relative to the fluorescence intensity ratio. Cutoff value = 5.5 (ratio for control)

6 onwards only in the first intensification phase. Finally, the re-induction phase comprises 4 administrations of L-Asp.

The patient received intravenously administered L-Asp (6,000 U/sqm) 9 times in the induction phase and 1 time (25,000 U/m²) in the first intensification phase without any adverse effects. Approximately 1 month later, in the second intensification phase, she complained of itching, nausea, and systemic urticaria during L-Asp (25,000 U/m²) drip infusion. An allergic response was identified, and L-Asp was discontinued. A few days later, a delayed-type hypersensitivity reaction developed in the form of blisters with erythema on the arm proximal to the catheter insertion site (Fig. 1). The absolute number of peripheral white blood cells was 2,330/μL (lymphocytes, 186/μL [8%], and neutrophils, 2,097 μL [90%]) when symptoms developed.

In the third intensification phase (11th administration of L-Asp), methylprednisolone, an histamine H1 receptor antagonist, and an histamine H2 receptor antagonist were administered to prevent allergic responses before and after L-Asp administration. However, symptoms identical to those observed in the second phase developed after 60% of the L-Asp protocol dose had been infused. After this episode, an increased amount of methylprednisolone (up to 125 mg/m²) and dexamethasone tapered over 3 days were administered to prevent early and delayed-type hypersensitivity. Mild erythema and induration were still observed at the proximal to the infusion site as a delayed-type hypersensitivity reaction several days after L-Asp administration in the subsequent phases. The plasma ammonia concentrations increased (maximum, 321 g/dL on the next day of L-Asp administration). The antithrombin III activity

and the level of fibrinogen decreased. The minimum antithrombin III activity was 90%, and the minimum fibrinogen was 113 mg/dL on the next day of L-Asp administration. Levels of immunoglobulin (Ig) E against L-Asp and of IgG against L-Asp were measured by Kyowa Hakko Kirin Co., Ltd. after the first and second allergic response episodes and were lower than normal (**Table 2**).

(Collaboration with Kyowa Hakko Kirin Co., Ltd., without any conflict of interest)

Discussion

Although L-Asp is an important reagent against acute lymphoblastic leukemia²⁶, its use may be limited because of allergic responses. Patients' immune systems recognize L-Asp as a foreign substance because it is a large enzyme (140 kD) and is derived from *E. coli*. Allergic reactions to L-Asp occur in 6% to 43% of cases³. Most reactions are immediate-type hypersensitivity reactions that can result in pyrexia, urticaria, bronchial asthma, and anaphylactic shock⁷. In contrast, delayed-type hypersensitivity in response to L-Asp administration is unusual. In our patient both immediate-type hypersensitivity and delayed-type hypersensitivity developed. The same symptoms of itching, erythema, blister formation, and induration occurred even when titers of anti-L-Asp IgE and IgG were not significantly elevated. In addition, blisters and induration were observed more than 48 hours after L-Asp administration and at the proximal to the infusion site. Usually, T lymphocytes play an important role in delayed-type hypersensitivity. Therefore, anergy is observed in patients with lymphocytopenia. In the present case, the absolute number of peripheral white blood cells was 2,330/ μ L (lymphocytes, 186/ μ L [8%], and neutrophils, 2,097 μ L [90%]) when symptoms developed. The lymphocyte count seemed too low to cause delayed-type hypersensitivity. However, even patients with lymphocytopenia due to human immunodeficiency virus infection can show delayed-type hypersensitivity responses, such as to tuberculin purified protein derivative⁸. Therefore, this delayed reaction was not considered to be a conflict type of

delayed hypersensitivity. These lesions were treated with chrobetazole salve wrap therapy. Additional methylprednisolone, tapered dexamethasone, and antihistamines to prevent hypersensitivity were presumed to be effective in preventing severe reactions. Measurement of ammonia concentrations can be useful for predicting the effects of L-Asp⁹. In the present case, the plasma ammonia concentration increased. Therefore, we concluded that L-Asp administration could be continued and would continue to be effective. However, care should be taken to avoid osteoporosis¹⁰, which can occur as a result of excessive administration of corticosteroids. In contrast, the cumulative corticosteroid dose is less related to a decrease in bone mineral density than is calcium intake¹¹. At any rate, the patient's bone density should be monitored, and a sufficient daily dietary intake of calcium should be encouraged.

In the TCCSG protocol, L-Asp is administered intravenously and subcutaneously; however, because quadriceps contracture developed after intramuscular injection in many children in the 1970s, this route is avoided in Japan. In contrast, hypersensitivity reactions occur in 6% to 43% patients after intravenous L-Asp infusion³. Therefore, L-Asp is now primarily administered via the intramuscular or subcutaneous route in other countries¹². Even in Japan, the intramuscular route should be used instead of the intravenous route.

In other countries, L-Asp derived from *Erwinia* and PEG are also used, but these reagents have not been approved in Japan. Many therapeutic clinical trials have used PEG L-Asp because it is thought to cause hypersensitivity less often. Furthermore, *Erwinia* L-Asp, which has the same therapeutic effects as *E. coli* L-Asp, is used when *E. coli* L-Asp cannot be used because of hypersensitivity. The acute lymphoblastic leukemia Berlin-Frankfurt-Münster trials have reported the use of *E. coli* L-Asp, PEG L-Asp, and *Erwinia* L-Asp for adaptation¹³. They suggested a relationship between efficacy and the anti-*E. coli* L-Asp antibody titer. As alternative chemotherapy reagents for acute lymphoblastic leukemia, PEG L-Asp and *Erwinia* L-Asp should be quickly approved for use in Japan.

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