

Anaphylactoid Reactions to *Escherichia Coli* and *Erwinia Asparaginase* in Children with Leukemia and Lymphoma

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The incidence and clinical characteristics of anaphylactoid reactions to intravenous asparaginase were assessed in 196 patients given *E. coli* asparaginase and 49 patients given *Erwinia* asparaginase. All patients were given a 50 IU intravenous test dose followed in 30 min by the full dosage (10,000 IU/m²), if no reaction occurred to the test dose. Twenty-nine of 196 patients (14.8%) given *E. coli* asparaginase had an anaphylactoid reaction, occurring after their first through 12th doses. The probability of an anaphylactoid reaction was significantly greater in those patients not receiving concomitant prednisone-vincristine and patients with a hiatus between courses of asparaginase therapy. By logistic regression analysis, other variables such as age, sex, race, diagnosis, total number of doses and concurrent methotrexate or arabinosylcytosine did not contribute significantly to the probability of a reaction. Twenty-three of the patients who had reacted to *E. coli* asparaginase and 26 patients who had not reacted to *E. coli* asparaginase were subsequently given *Erwinia asparaginase*. Seven of these 49 patients (14%) had an anaphylactoid reaction. The probability of a reaction to *Erwinia* asparaginase was significantly related to a prior reaction to *E. coli* asparaginase, concomitant prednisone-vincristine therapy, total number of asparaginase doses, number of prior *E. coli* asparaginase doses, and diagnosis, when assessed by a logistic regression model. However, after adjusting for prior reaction to *E. coli* asparaginase and the total number of asparaginase doses given, the other variables did not contribute significantly to the probability of a reaction. Only 5/29 patients reacting to *E. coli* asparaginase and 1/7 reacting to *Erwinia* asparaginase had a reaction to the test dose. None of the reactions were fatal.

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THE ENZYME L-asparaginase has been used clinically to treat leukemia and lymphoma for over ten years. Despite the extensive clinical experience with this drug, the optimal dosage and schedule of asparaginase remain undefined. Likewise, the characteristics and incidence of asparaginase toxicities have not been clearly established for the various treatment protocols and combination regimens containing this agent. One of the well

recognized and most common acute toxicities associated with administration of this bacterial enzyme are anaphylactoid reactions.¹⁻¹⁴ These immediate hypersensitivity reactions have been reported to occur in up to 33% of patients treated with *Escherichia coli* asparaginase.¹ Although an IgE-mediated mechanism has been proposed¹³ for these reactions, the precise immunological basis has not been fully elucidated. Therefore, the term "anaphylactoid" will be used herein.

Clinical experience with *Erwinia* asparaginase indicates that it can be administered without anaphylactoid reactions to patients who have had previous reactions to *E. coli* asparaginase.^{1,12} However, the combined incidence of anaphylactoid reactions to these two types of asparaginase has not been assessed in a large series of patients. Moreover, variables which influence the probability of anaphylactoid reactions have not been defined. The present study was undertaken to determine the proportion of patients having anaphylactoid reactions to either type asparaginase, to assess the clinical characteristics of these reactions, to identify clinical variables which influence the probability of anaphylac-

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toid reactions, and to determine the value of a 50 IU intravenous test dose in identifying patients sensitive to asparaginase.

Methods

All newly admitted children with leukemia or lymphoma given either *E. coli* L-asparaginase or *Erwinia carotovora* L-asparaginase from January 1976 through March 1979 were included in this study. *E. coli* asparaginase (Merck, Sharpe, Dome, NSC 109229) and *Erwinia* asparaginase (The Microbiological Research Establishment, NSC 106977) were supplied by the NCI. The records of these patients were retrospectively reviewed to determine the occurrence and characteristics of anaphylactoid reactions to asparaginase. The review was facilitated by a prospectively generated, computer-based record of all anaphylactoid reactions to asparaginase and all doses of all drugs administered to every patient at St. Jude Children's Research Hospital. The diagnosis and characteristics of all reactions were verified independently by two investigators.

For this study, anaphylactoid reactions to asparaginase were defined as the occurrence of any of the following signs or symptoms temporally related to the administration of asparaginase: hypotension (≥ 20 mm Hg drop in systolic blood pressure), respiratory distress (dyspnea and/or bronchospasm), tachycardia, urticaria and angioedema. These reactions were further subclassified as severe reactions if hypotension and/or respiratory distress occurred, or minor reactions when both respiratory distress and hypotension were absent. All reactions were immediately treated with intravenous antihistamine (diphenhydramine); severe reactions were also treated with intramuscular epinephrine. Patients with persistent bronchospasm or prolonged hypotension received intravenous corticosteroid therapy.

Fisher's exact test¹⁵ was used to determine the significance of differences in proportions of anaphylactoid reactions in subgroups of patients. A logistic regression model¹⁶ was used to relate the probability of an anaphylactoid reaction to multiple clinical and treatment variables.

Results

During the three-year study period, *E. coli* asparaginase was given to 196 newly admitted patients at St. Jude Children's Research Hospital. All of these patients were given an intravenous (bolus) 50 IU test dose of the respective asparaginase, followed in 30 min by the full intravenous asparaginase dosage of 10,000 IU/m² if no reaction to the test dose occurred. The full dose was administered by slow intravenous push over 30–60 min. Of these patients, 154 had a diagnosis of acute

TABLE 1. Outline of Concurrent Drug Therapy

I. Acute lymphocytic leukemia
Initial Remission Induction: (Randomized)
Group 1: Prednisone: 40 mg/m ² d orally for 28 days
Vincristine: 1.5 mg/m ² /wk IV (maximum per injection: 2.0 mg) on days 1, 8, 15 and 22
Daunomycin: 25 mg/m ² /wk IV on days 2, 8, 15 and 22
Induction failures after day 28;
Asparaginase 10,000 IU/m ² day 28 and 35
Arabinosylcytosine 300 mg/m ² day 28 & 35
Continue prednisone, vincristine
Group 2: Same as in Group 1, but add:
Asparaginase: 10,000 IU/m ² /wk on days 3 and 9
Group 3: Same as in Group 1 but if in remission after 28 days, follow with:
Asparaginase: 10,000 IU/m ² IV twice weekly for 4 doses
Arabinosylcytosine: 300 mg/m ² IV twice weekly for 4 doses
Reinduction Therapy: (First Hematologic Relapse)
Asparaginase 10,000 IU/m ² IV weekly
Arabinosylcytosine 300 mg/m ² IV weekly
Asparaginase 10,000 IU/m ² IV weekly $\times$ 2 (days 1 and 8)
Prednisone 40 mg/m ² /day in three divided doses $\times$ 28 days
Daunomycin 25 mg/m ² IV weekly $\times$ 4
II. Acute nonlymphocytic leukemia (first relapse)
Methotrexate 60 mg/m ² IV, day 1
Asparaginase 10,000 IU/m ² IV, day 2
(Repeat course every 1–2 weeks)
III. Non-Hodgkin's Lymphoma (in a leukemia phase)
Vincristine 0.75 mg/m ² IV every 12 hours $\times$ 2 doses, day 1
Methotrexate 60 mg/m ² IV, day 2
Asparaginase 10,000 IU/m ² IV, day 3
(Repeat course every 1–2 weeks)
IV. Chronic Myelocytic Leukemia
Methotrexate 100 mg/m ² IV, day 1
Asparaginase 10,000 IU/m ² IV, day 2
(Repeat course weekly $\times$ 2)

lymphocytic leukemia (ALL), 30 acute nonlymphocytic leukemia (ANLL), 11 non-Hodgkin's lymphoma (NHL), and one chronic myelocytic leukemia. There were 108 males and 88 females ranging in age from three month–19.5 years (median age, 6.5 years). Details of other chemotherapy administered concomitantly are summarized in Table 1.

A total of 620 *E. coli* asparaginase doses were administered to these 196 patients, ranging from 1–38 doses per patient (median, 3.2 doses per patient). Twenty-nine of these 196 patients had an anaphylactoid reaction. Thus, the overall incidence of anaphylactoid reactions to *E. coli* asparaginase was 14.8% of treated patients or a risk per dose of 4.7%. Six patients reacted to their first *E. coli* asparaginase dose, four to the second

TABLE 2. Clinical Characteristics of Anaphylactoid Reactions to Asparaginase

	E. Coli		Erwinia	
	With other manifestations	Alone	With other manifestations	Alone
Reaction characteristics				
Hypotension	4	0	1	0
Respiratory Distress	13	3	2	0
Urticaria	14	3	2	4
Angioedema	9	0	0	0
Tachycardia	4	0	0	0
Severe Reactions	15		2	
Minor Reactions	14		5	
Reaction to test-dose	5		1	
Reaction to < full dose	9		0	
Reaction to full dose	15		6	
Primary diagnosis				
ALL	18		2	
ANLL	8		3	
NHL	3		2	

dose, nine to the third dose, three to the fourth, two to the fifth, and one each to the sixth, seventh, eighth, tenth and twelfth doses. All reactions occurred within 30 min of asparaginase administration.

Twenty-three of the 29 patients allergic to *E. coli* asparaginase were subsequently administered *Erwinia* asparaginase (1-12 doses; median 3) and six (0.26) had an anaphylactoid reaction. Two of these patients reacted to their first *Erwinia* dose while the other four patients received from 2-7 doses of *Erwinia* asparaginase before having a reaction. Twenty six additional patients who had not reacted to 1-6 doses of *E. coli* asparaginase were given *Erwinia* asparaginase (1-6 doses) and one (0.04) patient had an anaphylactoid reaction. Thus, a total of 49 patients received 159 *Erwinia* asparaginase doses and seven (0.14) had an anaphylactoid reaction, or a risk per dose of 4.5% (7/159). All reactions occurred within 30 min of asparaginase administration.

As previously stated, all of these asparaginase doses were preceded by a 50 IU intravenous test dose of the respective asparaginase. Only five of the 29 patients (0.17) reacting to *E. coli* asparaginase had a reaction to the test dose while one of seven (0.14) patients reacting to *Erwinia* asparaginase reacted to the test dose.

Characteristics of the 36 anaphylactoid reactions to asparaginase are summarized in Table 2. The most frequent characteristic was urticaria, which was observed in 23 of the 36 reactions; while respiratory distress occurred in 18 cases. Hypotension, angioedema and tachycardia were observed less frequently and always occurred with other primary symptomatology. Additional

symptoms observed as part of the reaction complex included vomiting, nasal congestion, lacrimation, and abdominal pain. Using the previously described definition of severe and minor reactions (methods), 16 patients had 17 severe reactions (15 *E. coli*, two *Erwinia*) and 16 patients had 19 minor reactions (14 *E. coli*, five *Erwinia*). Two patients had both a severe and minor reaction, and appear in both groups.

A logistic regression model¹ was used to relate the probability of a reaction to *E. coli* asparaginase to the following variables: (1) number of *E. coli* asparaginase doses; (2) patient race; (3) patient sex; (4) patient age; (5) diagnosis; (6) concurrent prednisone-vincristine therapy; (7) concurrent methotrexate therapy; (8) concurrent arabinosylcytosine therapy; and (9) a hiatus of at least one month between courses of *E. coli* asparaginase therapy. When the probability of a reaction was compared to each variable independently, the most highly significant variables were concurrent prednisone ($\chi^2 = 11.57$) and concurrent vincristine ($\chi^2 = 11.49$). However, there was a high correlation of these two variables, with 92 of 96 patients receiving prednisone also receiving vincristine, and 92 of 103 patients receiving vincristine also receiving prednisone. Because of this high correlation, only those patients who received both prednisone and vincristine ($n = 92$) or those that received neither prednisone nor vincristine ($n = 89$) were included in the logistic regression analysis. However, it is of interest that among all patients given prednisone ($n = 96$), those also given vincristine ($n = 92$) had a significantly lower reaction rate (0.019 versus 0.75, $P = 0.00055$). Among those not given prednisone ($n = 100$), there was no significant difference between those given vincristine ($n = 11$) and those not given vincristine ($n = 89$) (0.36 versus 0.21, $P = 0.27$).

The results of the logistic regression analysis of the 181 evaluable patients are summarized in Table 3. The significance of the relationship between the probability of a reaction and the nine variables were determined by the likelihood ratio test.¹⁶ As shown, the only variables that correlated significantly to the probability of a reaction were the number of *E. coli* asparaginase doses, diagnosis, concurrent prednisone-vincristine, concurrent methotrexate, and the presence of a hiatus between courses of asparaginase therapy. However, after adjusting for the effects of concurrent prednisone-vincristine, and the presence of a hiatus between courses, the other variables became insignificant. Interactions were also considered between the most significant variable (concurrent prednisone-vincristine) and all other variables, and in no case was a statistically significant interaction found ($P > 0.1$ in all cases). Therefore, the

probability of a reaction depends most heavily on whether or not concurrent prednisone and vincristine are given, and the presence or absence of a hiatus between courses of therapy; given that when these variables are used the other variables do not add significantly to the relationship. From these data, the relationship can be characterized by the equation:

$$\ln \frac{p}{1-p} = -1.5 + 1.33H - 2.0PV$$

where p = the probability of a reaction to *E. coli* asparaginase, H = 1 if a hiatus is present and 0 if no hiatus, and PV = 1 if concurrent prednisone-vincristine are given and 0 if not.

A logistic regression model¹ was also used to relate the probability of an *Erwinia* asparaginase reaction to the following variables: (1) total number of asparaginase doses; (2) prior *E. coli* asparaginase reaction; (3) concomitant prednisone-vincristine therapy; (4) number of *Erwinia* asparaginase doses; (5) number of *E. coli* asparaginase doses; (6) diagnosis; and (7) time between course of *E. coli* and *Erwinia* asparaginase. The significance of the relationship between the probability of a reaction and these seven variables was determined by the likelihood ratio test.¹⁶ As summarized in Table 4, the probability of a reaction was significantly related to variables 1, 2, 3, 5 and 6. However, after adjusting for prior reactions to *E. coli* asparaginase and total number of asparaginase doses, the effects of other variables such as diagnosis, prednisone-vincristine therapy, and number of prior *E. coli* asparaginase doses were not significant. From these data, the relationship can be characterized by the equation:

$$\ln \frac{p}{1-p} = -4.87 + 0.262D + 1.70PR$$

where p = the probability of a reaction to *Erwinia* asparaginase, D = the total number of asparaginase doses given, and PR = 1 if a prior reaction to *E. coli* asparaginase has occurred or PR = 0 if no prior reaction.

Discussion

The incidence of immediate hypersensitivity reactions to *E. coli* asparaginase varies widely in published studies¹⁻¹⁴ ranging from 5-33% of treated patients. When all patients reported in these published studies and the 196 patients described in the present study are combined, there have been 284 "allergic reactions" in 1442 patients, or an incidence of 19.7% of treated patients. This overall incidence of "allergic reactions" is similar to the 15% incidence of anaphylactoid reactions

TABLE 3. Analysis of the Probability of an Anaphylactoid Reaction to *E. Coli*: Asparaginase by Logistic Regression

Variable #	Variable	Likelihood ratio test	Degrees of freedom	Significance*
(1)	Total number of <i>E. coli</i> asparaginase doses	4.60	1	S
(2)	Patient race	2.54	1	NS
(3)	Patient sex	1.03	1	NS
(4)	Patient age	0.32	1	NS
(5)	Diagnosis (ALL, Non-ALL)	5.09	1	S
(6)	Concurrent prednisone-vincristine	15.20	1	S
(7)	Concurrent methotrexate	5.09	1	S
(8)	Concurrent cytosine arabinoside	3.74	1	NS
(9)	Hiatus >1 month between courses	5.8	1	S
(6, 9)		19.08	2	S
(6, 9, 1)		19.3	3	NS
(6, 9, 2)		19.8	3	NS
(6, 9, 3)		19.57	3	NS
(6, 9, 4)		19.53	3	NS
(6, 9, 5)		20.4	3	NS
(6, 9, 7)		20.22	3	NS
(6, 9, 8)		20.22	3	NS

* S = significant at the 0.05 level; NS = not significant. In considering multiple variables (S, NS) indicates whether the last variable considered adds significantly to the previous variables.

observed in our patients. However, it is apparent from these studies that several variables may influence the incidence of anaphylactoid reactions to asparaginase.

It has been suggested that the risk of "hypersensitivity reactions" increases with continuing administration of asparaginase or with readministration following a period of no asparaginase therapy.^{1,6} Oettgen *et al.*¹¹ report that the risk of reactions is dose-related, while Jaffe *et al.*⁴ suggest that the risk is unrelated to dose or schedule. Nesbit *et al.*⁷ have reported that the concomitant administration of 6-mercaptopurine significantly reduces the incidence of allergic reactions when

TABLE 4. Analysis of the Probability of an Anaphylactoid Reaction to *Erwinia* Asparaginase by Logistic Regression

Variable #	Variable	Likelihood ratio test	Degrees of freedom	Significance*
(1)	Total number of asparaginase doses	7.07	1	S
(2)	Prior <i>E. coli</i> asparaginase reaction	5.31	1	S
(3)	Prednisone-vincristine therapy	3.88	1	S
(4)	Number of <i>Erwinia</i> asparaginase doses	1.73	1	NS
(5)	Number of <i>E. coli</i> asparaginase doses	4.77	1	S
(6)	Diagnosis (Non-ALL vs. ALL)	8.33	1	S
(7)	Time between asparaginase courses	0.01	1	NS

* S = significant at the 0.05 level; NS = not significant.

compared to patients given asparaginase alone (5.1% versus 27%). Land *et al.*³ reported a trend toward fewer reactions in patients receiving concurrent prednisone-vincristine, although the difference was not statistically significant (7% versus 14%; $P > 0.05$). A similar observation described by Jacquiullatt *et al.*³ was also not statistically confirmed. Conversely, Jaffe *et al.*⁴ and Dellinger *et al.*¹ have reported that concurrent prednisone therapy or pretreatment with corticosteroids does not avert asparaginase reactions.

The Childrens Cancer Study Group (CCSG)¹⁰ compared the efficacy and toxicity of intravenous (IV) versus intramuscular (IM) asparaginase as a single agent for treatment of acute leukemia relapse in 153 children. Using a three-times-weekly schedule, response rates were comparable with the two routes of administration and overall toxicity was lower with IM asparaginase. The incidence of "sensitivity reactions" was 37.5% with IV asparaginase and 31.5% with IM administration. However, when patients experiencing only abdominal pain and/or fever are excluded, reactions occurred in 28.8% with IV asparaginase and 12.3% with IM. The 28.8% incidence of "sensitivity reactions" to IV asparaginase in this group of patients is comparable to the 23% incidence observed in our patients not receiving prednisone-vincristine. The 12.3% incidence observed¹⁰ with IM asparaginase in the absence of prednisone-vincristine therapy is intermediate to the 6.3% incidence observed in our patients receiving prednisone-vincristine and the 23% in our patients not receiving prednisone-vincristine. An even lower incidence of anaphylactoid reactions if IM asparaginase is given with prednisone-vincristine is an attractive possibility. The fact that the most severe category of sensitivity reactions ("anaphylaxis") was not observed with IM asparaginase in the CCSG study¹⁰ is provocative, but awaits confirmation.

All investigators^{4,6,11-13,16} assessing the value of pretreatment skin tests agree that this is not a reliable means of predicting hypersensitivity reactions. There is also general agreement that fatal reactions are rare, being reported in only two¹⁷ of 1737 patients described in the published literature reviewed herein. Published data on hypersensitivity reactions to *Erwinia* asparaginase are much less extensive, precluding determination of the incidence and risk factors from previous studies.

The data derived from our study provide clarification of several points regarding the incidence and risk factors of anaphylactoid reactions to *E. coli* and *Erwinia* asparaginase. First, the incidence of anaphylactoid reactions to *E. coli* asparaginase is about 15% of treated patients, although subgroups of these patients are at significantly greater risk. It was evident that the risk of an anaphylactoid reaction to *E. coli* asparaginase

was significantly greater in patients who were not receiving concomitant prednisone-vincristine therapy and patient who had a hiatus of at least one month between courses of *E. coli* asparaginase. Furthermore, it was observed that the risk of anaphylactoid reactions to *Erwinia* asparaginase was significantly greater in patients who had previously reacted to *E. coli* asparaginase, and that the risk of a reaction was related to the total number of asparaginase doses given. The greater risk of *Erwinia* anaphylactoid reactions in patients previously reacting to *E. coli* asparaginase is in contrast to previous reports of no cross-reactivity.² However, the increased reaction rate to *Erwinia* asparaginase in those patients with prior reactions to *E. coli* asparaginase must be interpreted cautiously, since these patients may have been more sensitive to all foreign antigens. The lower risk of anaphylactoid reactions to either asparaginase in ALL patients was apparently due to fewer variables associated with increased risk of a reaction in this group; specifically the smaller number of asparaginase doses given ALL patients, the greater proportion of ALL patients receiving concomitant prednisone-vincristine, and the smaller proportion of ALL patients with prior reactions of *E. coli* asparaginase.

This study has also demonstrated that a 50 IU intravenous test dose of asparaginase rarely results in a reaction (<1%) and yields false negative results in more than 80% of patients who have an anaphylactoid reaction. Thus, the use of a 50 IU IV test dose is no better than previously reported results using interdermal skin tests.

Although anaphylactoid reactions to asparaginase may be fatal, none of the reactions in the present study had a fatal outcome. The lack of fatal reactions in this as well as other studies may have been due to careful observation of all patients and rapid treatment of those patients manifesting anaphylactoid reactions. Results of the present study should be of value in minimizing the risk of anaphylactoid reactions in future clinical trials with chemotherapy regimens containing asparaginase.

ADDENDUM

The equations for calculating the probability of an anaphylactoid reaction to *E. Coli* or *Erwinia* Asparaginase can be solved as follows (note abbreviations in text):

E. coli Asp.:

$$p = \frac{e^{(-1.5 + 1.33H - 2.0PV)}}{1 + e^{(-1.5 + 1.33H - 2.0PV)}}$$

Erwinia Asp.:

$$p = \frac{e^{(-4.87 + 0.262D + 1.7PR)}}{1 + e^{(-4.87 + 0.262D + 1.7PR)}}$$

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