

Does heparin prophylaxis prevent exacerbations of hereditary angioedema?

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Background: Hereditary angioedema (HAE) is a rare disorder characterized by episodes of angioedema of the skin, mucous membranes, and gastrointestinal tract resulting from a defect in the gene that produces C1 esterase inhibitor. Although in vitro laboratory data and past reports suggested that heparin might be efficacious in preventing HAE attacks, no controlled study has been reported to examine heparin's efficacy in this regard.

Objectives: We sought to determine the safety and efficacy of inhaled and subcutaneous heparin versus that of placebo in the prevention of HAE attacks.

Methods: We performed a double-blind, double-dummy, saline placebo-controlled, randomized, 3-way crossover study with 11 visits.

Results: The study was designed to enroll 24 patients. Twenty-two patients were randomized and received the study drug. Patients did not have a significant decrease in average flare intensity after they received injected or inhaled heparin compared with that seen after placebo, the primary endpoint. However, when patients received injected heparin, they had a statistically significant decrease in average flare intensity compared with that seen with inhaled heparin after a normalizing transformation was applied. When the means are back transformed, this translates into median flare intensities of 9.2, 8.0, and 5.1 in the patients treated with inhaled heparin, placebo, and injected heparin, respectively. There were no significant differences when individual symptoms were examined, when total numbers of flares over a 6-week observation period were examined, or when global evaluations by the patients and investigators were evaluated. Adverse event severity was fairly uniform across treatments, with the majority of events classified as moderate and the remainder split between mild and severe. Injected heparin treatment was associated with higher rates of relatedness than other treatments, which was partially explained by 17 adverse events specifically related to the injection process itself (tenderness, bruising, redness, pain, and itching at the injection site). The injection treatment was also associated with a larger overall number of reported adverse events (70 vs 48 in the placebo treatment). Tenderness and bruising at the injection site were entirely confined to the injected heparin treatment.

Conclusions: Injected and inhaled heparin failed to attenuate average flare intensity, the primary endpoint, compared with placebo. Interestingly, after patients injected heparin, they had a significant decrease in average flare intensity compared with that seen after inhalation of heparin. There were no differences among groups in other efficacy parameters. Taken together, these data indicate that commercial heparin was ineffective in preventing exacerbations of HAE. (*J Allergy Clin Immunol* 2002;109:995-1000.)

Key words: Hereditary angioedema, heparin, C1 esterase inhibitor

Hereditary angioedema (HAE) is a rare disorder characterized by episodes of nonpruritic angioedema of the skin, mucous membranes, and gastrointestinal tract resulting from a defect in the gene that produces C1 esterase inhibitor (C1INH).¹⁻⁵ Typically, patients with HAE might develop attacks of angioedema of the face, torso, and extremities and incapacitating abdominal pain and have laryngeal edema. Episodes might progress rapidly, leading to asphyxia and fatal laryngeal obstruction. Unfortunately, the diagnosis of HAE is often not made until many years after symptoms begin, and published cases suggest a case fatality rate of 25%, with death being caused by respiratory obstruction.^{1,4}

Since the 1970s, attenuated androgens have been administered chronically to prevent attacks of HAE, presumably by increasing the levels of C1INH.^{6,7} However, attacks might still occur in patients treated with these agents. Moreover, attenuated androgens are controlled substances that might cause unacceptable adverse events, such as virilization, decreases in high-density lipoprotein cholesterol levels, and liver disease, which might preclude their use. Currently, there is no Food and Drug Administration–approved therapy in the United States for the treatment of acute flares of HAE. In Europe C1INH concentrate is approved for the treatment of acute exacerbations of HAE,⁸ but replacement therapy is expensive and potentially might transmit viral agents, such as HIV and hepatitis. Thus there remains an urgent need for a safe and effective therapy that can be administered chronically to patients with HAE to prevent attacks.

Two previous reports described the safety and possible efficacy of heparin in patients with HAE.⁹⁻¹⁰ Levine and Stechschulte reported the use of open label inhaled heparin for six weeks in two patients with HAE.¹⁰ The first patient typically had attacks of HAE about every week when not treated with stanozolol. She became asymptomatic while receiving the heparin, had recurrence of symptoms when therapy was stopped, and again became asymptomatic when the heparin therapy was reinstituted. Both C4 and C1INH levels remained low, even

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Abbreviations used

C1INH: C1 esterase inhibitor
 HAE: Hereditary angioedema
 PT: Prothrombin time
 PTT: Partial thromboplastin time

during therapy, when the patient was asymptomatic. The other patient studied by Levine and Stechschulte had attacks of HAE about every 2 weeks when not treated with stanazolol, and when heparin therapy was instituted, she became asymptomatic.¹⁰ It is interesting that Levine and Stechschulte observed a slight but not statistically significant decrease in both C4 and C1INH levels¹⁰ when they examined the effect of parenteral heparin administration in patients who did not have HAE.

Although these reports suggested that heparin might be efficacious in preventing attacks, no controlled study has been reported. A properly controlled study has been necessary to examine whether this relatively safe, inexpensive, and readily available substance might be used to prevent attacks of HAE. This report describes a controlled study that was designed to determine whether heparin administered by means of inhalation or by means of a subcutaneous route is efficacious and safe compared with placebo in the prevention of HAE exacerbations.

METHODS

Patients

Patients with a history of HAE were recruited from across the United States. A total of 31 patients provided written informed consent: 3 were considered screen failures, and 28 qualified for enrollment into the study. Six patients withdrew consent before randomization, and therefore 22 patients were randomized and treated with study medication. Three patients withdrew consent after randomization, and 3 were discontinued because of noncompliance.

Each patient must have had a confirmed diagnosis of HAE on the basis of a low C4 antigenic level, a positive family history, and a low C1INH antigenic level (type I HAE) or C1INH functional level (type II HAE). Functional C1 must have been within normal levels if the diagnosis of acquired C1INH was possible. A negative family history did not exclude a patient when the patient met all other inclusion and exclusion criteria, had a normal C1 level, and had no evidence of acquired C1INH deficiency. Each patient must have had a history of symptoms of HAE, including episodic swelling, difficulty breathing, or abdominal pain that was believed by the investigator to be part of the HAE syndrome and to require therapy. Symptoms must have been of sufficient frequency and severity to allow evaluation during a 6-week evaluation period. In the opinion of the investigator, a minimum of 1 to 2 episodes of HAE (of at least mild intensity) was to be expected during a 6-week time interval on the basis of past history.

The study was approved by the University of Iowa Institutional Review Board.

Study design

The study was designed to examine the safety and efficacy of inhaled and subcutaneously administered heparin compared with that of matched placebo in as many as 24 patients with HAE in a double-blind, double-dummy, saline placebo-controlled, randomized, 3-way crossover trial over 7 months. Treatment periods were labeled as phase I, phase II, and phase III.

There were 11 encounters during the course of the study: encounter 0 (screening), encounter 1 (beginning of phase I), encounters 2 and 3 (interim evaluations), encounter 4 (end of phase I and beginning of phase II), encounters 5 and 6 (interim evaluations), encounter 7 (end of phase II and beginning of phase III), encounters 8 and 9 (interim evaluations), and encounter 10 (final visit).

Procedures

Patients received an initial evaluation at encounter 0. After written informed consent was obtained from the patient or legal guardian, the following interventions were performed: (1) medical history, medication history, and evaluation of inclusion-exclusion criteria; (2) complete physical examination; (3) vital signs measurement; (4) chest radiography if not obtained within the past 6 months; (5) electrocardiography; (6) urinalysis; (7) blood draw for complete blood count with differential and platelet count, prothrombin time (PT), activated partial thromboplastin time (PTT), chemistry screen, CH50, C3 antigen, C4 antigen, and C1INH antigen measurement; (8) blood draw for C1 functional level and C1INH functional level measurement if required to make the diagnosis of HAE; and (9) spirometry (FEV₁ and forced vital capacity measurement). If the patient met all of the criteria (except for the processing of the laboratory tests, for which the results were pending) he or she was instructed on the use of the diary cards. The patient was told to record symptoms and all medication use daily for the following 4 weeks (until encounter 1) and to continue to take regularly scheduled medication with no change in dose or frequency. The patient was then scheduled for encounter 1, which occurred approximately 1 month after the screening encounter.

At encounter 1, the diary cards were examined to determine whether the patient was compliant in providing a daily record of symptoms and medications taken and to determine that there were at least 1 to 2 exacerbations of HAE. Patients were then randomized and began phase I of the study. They were given a supply of study drug for inhalation and for subcutaneous injection and were instructed on proper medication administration. Blood was obtained for complete blood count with differential and platelet count and PT, PTT, CH50, C3, C4, and C1INH measurement. Vitals signs and adverse events were assessed. Patients were monitored daily (in the University of Iowa Clinical Research Center) after beginning treatment to assess safety (PT, PTT, adverse events, and vital signs) for up to a week after initiation of the study drug. Follow-up encounters (encounters 2 and 3) occurred at the end of the second and fourth weeks (locally or at the study site) and at the end of the second month (encounter 4). Patients evaluated symptoms throughout this period on a daily basis and globally at the end of the 2-month drug-administration period. The investigator also rated symptoms globally at the end of the 2-month drug-administration period. Additional laboratory tests and clinical evaluations were performed on a biweekly basis during the first month and on a monthly basis during the second month. This same procedure was repeated for phase II and phase III. Only symptoms occurring during the final 6 weeks of each 2-month study period were included in the efficacy evaluation to allow for a washout period. Thus each patient who completed the study received 2 months each of therapy with subcutaneously injected heparin, inhaled heparin, and placebo.

Study drug

A single lot of freeze-dried calcium heparin (160,000,000 units) was obtained from Celsus Laboratories (Cincinnati, Ohio). Heparin Calcium USP Lyophilized (lot HC-12893) from porcine intestinal mucosa was GMP manufactured by Celsus Laboratories pursuant to US Food and Drug Administration Drug Master File 7944 and 7951

by using a process that has been validated for elimination and inactivation of all detectable traces of input virus infectivity. Celsus Laboratories provided stability data for lot HC-12893 consisting of potency and pH, determined biannually over 5 years, to support a shelf life of more than 5 years. The heparin was then adjusted and packaged by the Pharmaceutical Services Facility in the University of Iowa College of Pharmacy to a concentration of 80,000 units in 4 mL for inhalation and 10,000 units in 1 mL for injection. Identically appearing syringes containing placebo were prepared. Syringes were examined for sterility, and the heparin was tested before and after being placed into the syringes for functional activity to determine the stability of the heparin in the syringes. The Pharmaceutical Services Facility performed testing for sterility and pyrogenicity; functional assays to ensure drug activity were performed by Celsus Laboratories.

Subcutaneously injected study drug. Calcium heparin (200 U/kg; maximum of 10,000 units) or matching placebo was administered subcutaneously every 12 hours. Syringes were adjusted as necessary by study personnel before dispensing the medication so that the patient received the proper dose of medication.

Inhaled study drug. Study drug or matching placebo was inhaled at the same approximate time of day during the entire study by using a model 646 DeVilbiss nebulizer and a nebulizer machine. Identical syringes containing 4 mL of saline or heparin in 4 mL of saline were poured into the nebulizer and administered slowly over 5 minutes. Then 4 mL of saline was added to the nebulizer and administered to remove all of the remaining heparin or placebo. The nebulizer was cleaned with disinfectant solution, allowed to dry, and stored in a clean place. Each patient who weighed less than 80 kg had the syringes (active drug and placebo) adjusted by study personnel so that the patient administered approximately 1000 units of the calcium heparin per kilogram of body weight (maximum of 80,000 units).

A record of drug use was kept by the patient and matched with used and unused medication syringes at the next clinic encounter. Compliance was determined on the basis of what the patient recorded on the medication records.

Evaluation criteria

Patients evaluated swelling, difficulty breathing, and abdominal pain on an 11-point scale (0-10) on a daily basis. The following units of measurement were derived from these symptoms.

Average flare intensity (ie, severity-days per flare). This was the average area under the curve for each flare during the interval of the last 6 weeks of each treatment period. The primary endpoint was average flare intensity for all 3 symptoms combined (analyzing the maximum of swelling, difficulty breathing, and abdominal pain). Individual symptoms (swelling, difficulty breathing, and abdominal pain) were secondary endpoints.

Total number of flares (ie, number of discrete flares). This was a count of discrete flares during the interval of the last 6 weeks of each treatment period. This was a secondary endpoint.

Total flare intensity (ie, total severity-days for all flares). This was the area under the curve for all flares during the interval of the last 6 weeks of each treatment period. This was a secondary endpoint.

Additional secondary efficacy endpoints were global evaluation by patient and global evaluation by investigator. Safety endpoints were vital signs, laboratory values, and adverse events.

Statistical methods

The primary analysis was an intent-to-treat analysis. The primary efficacy analysis used the linear models approach for crossover designs, with no carryover. Pairwise differences among the 3 treatments were examined with the Tukey multiple comparison procedure.

The secondary endpoints were analyzed with the same techniques described for the primary efficacy analyses. The Box-Cox method was used to identify normalizing, variance-stabilizing transformations for the primary and secondary measures. Means and mean differences were computed on the transformed scales and on the original scales.

RESULTS

Demographics

Of the 22 randomized patients (6 male and 16 female patients, intent-to-treat population), 19 successfully completed phase I, 16 completed phases I and II, and 15 (5 male and 10 female patients) successfully completed all phases and were considered *protocol correct*. The average age of the 22 randomized intent-to-treat patients was 32.6 years; 21 were white, and 1 was Hispanic (Table I).

Compliance varied from 100% to a low of 90.4%. In a few cases (2 phases for one patient and 3 phases for another patient) records were lost by the patients, and therefore it was impossible to determine compliance with medication use.

Efficacy results

When patients received injected heparin, they had a decrease in the severity of flares (measured as a decrease in the average flare intensity) compared with when they received either inhaled heparin or placebo (Table II). However, only the difference in average flare intensity between the groups receiving injected heparin and inhaled heparin was statistically significant. The contrasts between injected heparin and placebo and between inhaled heparin and placebo were the planned contrast for the primary endpoint, and thus this study must be reported as having a negative result.

When we examined the back-transformed means, this translated into median flare intensities of 9.2, 8.0, and 5.1 in the patients treated with inhaled heparin, placebo, and injected heparin, respectively. Clearly, the average flare intensity was slightly higher after inhaled heparin than after placebo (although this difference, again, was not statistically significant).

There were no other significant differences when individual symptoms were examined or when total numbers of flares or the total flare intensity over the 6-week observation period were examined. Also, there were no significant differences in the global scores by the investigator or patients.

Safety results

Adverse events were distributed uniformly across treatments, with the majority of events classified as moderate and the remainder split between mild and severe. The split between mild and severe was not always even, but the highest rate of severe classifications (ie, 23% in the injection treatment period) was matched by an identical percentage in the placebo treatment. The injected heparin treatment was associated with higher rates of relatedness than other treatments, which can be partially explained by

TABLE I. Demographic data

Protocol correct (Y/N)	Sex	Age (y)	Ethnic origin
Y	F	8	White
Y	M	31	White
Y	M	28	White
Y	F	23	White
Y	F	44	White
Y	F	46	White
N	M	31	White
Y	M	60	White
Y	F	48	White
N	F	17	White
N	F	28	White
Y	F	74	Hispanic
N	F	23	White
Y	M	20	White
Y	F	14	White
Y	F	35	White
N	F	45	White
N	F	24	White
Y	F	39	White
Y	M	13	White
Y	F	27	White
N	F	39	White

TABLE II. Treatment contrasts for the primary endpoint: Average flare intensity for swelling, difficulty breathing, and abdominal pain (transformed)

Contrast	Transformed estimate	Tukey P value
Intent-to-treat		
Injected heparin vs placebo	-0.09164	.1007
Injected heparin vs inhaled heparin	-0.1237	.0249*
Placebo vs inhaled heparin	-0.03201	.7536
Protocol correct		
Injected heparin vs placebo	-0.1028	.0691
Injected heparin vs inhaled heparin	-0.1363	.0140*
Placebo vs inhaled heparin	-0.03353	.7362

* $P \leq .05$.

17 adverse events specifically related to the injection process (tenderness, bruising, redness, pain, and itching at the injection site). The injection treatment was also associated with a larger overall number of reported adverse events (70 vs 48 in the placebo treatment). This increase in adverse events can, again, be partially ascribed to a high rate of adverse events associated with the injection itself.

The most common adverse events (experienced on 5 or more occasions) were headache, tenderness or bruising at the injection site (discussed above), upper respiratory tract infections, knee pain or stiffness, sore throat, and bruising of various body parts. A single subject reported 6 of the 15 headaches, and injected heparin and placebo treatments were associated with the largest numbers of headaches (5 and 6, respectively). Tenderness and bruising at the injection site were entirely confined to the patients undergoing injected heparin treatment. Upper respiratory tract infections were relatively evenly spread across treatments. Subjects in the inhala-

tion phase of the study reported a relatively large number (7) of respiratory adverse events (3 sore throat and 1 each of sinusitis, nonproductive cough, strep throat, and bloody nose) compared with 2 with placebo (sore throat and tonsillitis), 1 with injected heparin (bloody nose), and 3 during prerandomization (sore throat, sinus infection, and tonsillitis).

DISCUSSION

More than 60 years ago, Ecker and Gross¹¹ first suggested that heparin might regulate complement activity. Since then, in vitro and in vivo studies in many laboratories have clearly shown that heparin acts at multiple steps in the complement cascade.¹²⁻²⁴ Heparin is a naturally occurring proteoglycan²⁵ produced by mast cells and basophils. Chains are highly sulfated, making heparin one of the most acidic polymers in the human body and, under physiologic conditions, a highly charged polyanion that reacts with a large number of substances.²⁶ Although heparin has primarily been recognized for its capacity to bind antithrombin III, it is more appropriate to consider heparin as a polyelectrolytic drug that has multiple biologic activities, including the ability to regulate complement activity in vitro.¹²⁻²⁹

Heparin might act in vitro on the classical pathway of complement,¹⁴⁻¹⁷ potentiating the action of C1INH,^{12,30-32} interfering with C1q binding to immune complexes,¹⁵ inhibiting C1 esterase interaction with C4 and C2 and C2 binding to C4,^{16,17} and suppressing reactive lysis.¹³ In addition, both native proteoglycan and commercial heparin have been shown to act in vitro to inhibit generation of cell-bound and fluid-phase alternative pathway convertase, to inhibit preformed alternative pathway convertase, and to impair factor H-mediated decay of the alternative pathway convertase.¹⁸⁻²⁰ Heparin also augments factor H activity.²¹⁻²³ Furthermore, studies show that heparin might exert activity on complement in whole serum and in vivo.^{33,34}

The first suggestions that heparin might be used to treat HAE were made in the 1970s, but heparin therapy was not examined at that time.³⁵ Two groups, first independently, demonstrated the ability of heparin to augment C1INH activity in vitro.^{30,31} Rent et al³⁰ showed that heparin potentiates C1INH activity in plasma, as well as in a system using purified proteins at concentrations much lower than those required to inhibit C1, and Nagaki and Inai³¹ demonstrated that heparin augments C1INH activity on C1 esterase but not on plasmin. Other glycosaminoglycans also have the capacity to augment the activity of C1INH but at higher concentrations than required for heparin. Kinetic studies reveal that heparin augments C1INH activity by 15-36 to 35-fold.³⁷ Caughman et al³² observed an anodal shift in electrophoretic mobility of C1INH in the presence of heparin. They also reported that heparin inhibits heat-aggregated IgG activation of complement in normal, but not in C1INH-depleted, human serum, suggesting to them that heparin's predominant activity on the classical pathway is through augmentation

TABLE III. Post hoc power analysis

No. of patients	Tukey			Unadjusted		
	Injected vs placebo	Injected vs inhaled	Injected vs other	Injected vs placebo	Injected vs inhaled	Injected vs other
12	0.09	0.32	0.25	0.17	0.48	0.39
18	0.14	0.51	0.40	0.24	0.66	0.56
24	0.19	0.66	0.53	0.31	0.79	0.69
30	0.24	0.78	0.65	0.37	0.88	0.78
36	0.29	0.86	0.75	0.44		0.86
42	0.35		0.82	0.50		

Power analysis for the indicated contrasts is shown. Power is shown for Tukey and unadjusted *t* tests. The treatment means, variances, and correlations are assumed to be those obtained in the intent-to-treat analysis of the primary endpoint in the present study.

Injected vs other. Contrast between injected heparin and average of placebo and inhaled heparin.

of C1INH. Hortin and Trimpe³⁸ demonstrated that a given concentration of heparin enhances the activity of C1INH on purified C1 esterase by 25-fold, but when the C1 esterase is present in the intact C1 macromolecule, heparin enhances C1INH activity by only 5-fold.

The present report suggests that patients who received heparin injected subcutaneously had significantly less severe flares of HAE than when they received inhaled heparin. However, the differences between heparin injected subcutaneously and placebo did not reach significance, perhaps because of the lack of power of this study. On this basis, the study shows lack of efficacy for heparin in the prevention of attacks of HAE, even though there were trends that suggested that a difference could have been seen if more patients had been studied. Also, there were no differences or trends when we examined the total numbers of flares, total flare intensity, or global evaluations by patient or by investigator. Moreover, we observed no differences among treatments when we examined individual symptoms (swelling, breathing difficulty, and abdominal pain).

Table III displays a post hoc power analysis based on the data from this study. The assumptions are that the treatment means and effect sizes are as estimated in the intent-to-treat analysis of the primary endpoint and that the same crossover design would be used for a follow-up study. If inhaled heparin is equivalent to placebo, then the true effect size is probably more realistically estimated by the "heparin injected" versus "other" contrast (placebo and inhaled heparin). Using conventional statistical practice (unadjusted comparisons for a planned contrast) and assuming that the placebo mean is best estimated by the average of placebo and injected heparin, a design in 30 patients will have about 78% power to detect an effect of the size observed in this study.

Most adverse events appeared to have been random occurrences and, with the exception of those related to local effects of injected heparin, were not related to treatment. Although adverse events were uniformly distributed across treatments, this study provides no information about the risks of receiving heparin for long periods of time. Other studies suggest that injection of heparin is associated with risks over longer periods of administra-

tion that would have to have been considered if heparin had been efficacious.

We conclude that the present study failed to demonstrate that injected heparin was efficacious in the prevention of attacks of HAE. Clearly, patients who received heparin by inhalation fared even worse than those who received placebo, and we must also conclude that inhaled heparin lacked any efficacy in preventing attacks of HAE.

If a follow-up study were to be conducted, then it would make the most sense to examine low-molecular-weight heparin because low-molecular-weight heparin is a safer product. Moreover, studies in our laboratory have shown that low-molecular-weight heparin has even more activity than commercial heparin in augmenting C1INH activity *in vitro*.¹² Until a study showing efficacy for low-molecular-weight heparin is conducted, we caution against the use of heparin to prevent attacks of HAE.

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