

Acute urticaria caused by subcutaneous recombinant hirudin: Evidence for an IgG-mediated hypersensitivity reaction

Andreas J. Bircher, MD,^a Czeslaw H. Czendlik, MD,^b
Sabine Langauer Messmer, MD,^a Philipp Müller, MD,^b and
Hans Howald, MD^b Basle, Switzerland

Extracts from the salivary glands of the blood-sucking leech *Hirudo medicinalis* are used in topical medications to treat venous disorders. Contact allergy to crude leech extract, which contains hirudin and other proteins such as eglins, bdellins, and hyaluronidase has been reported.^{1,2} Hirudin is a potent selective thrombin inhibitor and may therefore be of value in the treatment of thrombotic diseases.³ Recombinant desulfato-hirudin (rH), produced in *Saccharomyces cerevisiae* cultures, has a molecular weight of 6964 d and is practically identical to the natural hirudin variant 1, lacking only the sulfate moiety on tyrosine 63.⁴ Several pharmacologic and clinical trials with rH have been performed, so far with few adverse experiences such as hypersensitivity reactions⁴ or hemorrhages.⁵ We report a volunteer participating in Clinical Pharmacology studies with rH who experienced acute, probably IgG-mediated, urticaria on reexposure to hirudin.

CASE REPORT

A 30-year-old healthy man participated in several Clinical Pharmacology studies with rH. In 1989 he received two, and 2 months later, three intravenous injections of rH without adverse effects. Results of prick and intradermal tests with rH (up to 1 mg/ml) were negative before and 1 month after these investigations. Five years later, he received 15 mg of rH subcutaneously without adverse event. One week later at his seventh exposure, again 15 mg of rH was administered subcutaneously into the abdominal wall. Approximately 50 minutes later, he started to have a warm and prickling cutaneous sensation, and then generalized urticaria de-

Abbreviation used

rH: Recombinant desulfato-hirudin

veloped. His face was swollen, and he felt a slight respiratory oppression without signs of bronchospasm. There were no other local or systemic signs or symptoms, and in particular, no reaction at the injection site. Fifty milligrams of prednisolone-21-sodium-tetrahydrophthalate was immediately administered intravenously, and the symptoms subsided within 15 minutes. He was referred for an allergological workup 4 weeks after the event.

Allergological investigations

The subject had no history of atopic diseases or previous drug reactions. Prick tests with common respiratory allergens revealed a sensitization to grass and rye pollens; results of an IgE screening test were positive (SX-1, 2.7 kU/L, class 2) and total IgE 44 kU/L (normal, <100 kU/L). Results of skin prick and intracutaneous tests with *Candida* species and *S. cerevisiae* were negative. Initially, skin prick tests with rH, 15 mg/ml, were performed. The reaction produced a wheal of 2 mm and a flare of 6 mm, the histamine control (1 mg/ml) produced a wheal of 4 mm and a flare of 10 mm, and the vehicle control was negative. Intradermal tests at 0.00015 mg/ml produced a wheal/flare of 8 mm/10 mm, and at 0.0015 mg/ml, a wheal/flare of 10 mm/35 mm; whereas the reaction to histamine (0.1 mg/ml) was a wheal/flare of 15 mm/35 mm. The results of the prick test and the lower intradermal concentration were interpreted as equivocal; at 0.0015 mg/ml it was considered positive. No delayed or late skin test reactions occurred. Two unexposed control subjects and three rH-exposed control subjects had negative prick test and negative intradermal test results up to 1.5 mg/ml.

Specific IgE antibodies to rH were not detectable by the RAST method in the patient and the three exposed control subjects, and specific IgE antibodies to *Candida* species and *S. cerevisiae* were less than 0.35 kU/L (class 0). Determination of tryptase, a mast cell enzyme, at 1, 2, 3, 6, and 10 hours was less than 2 U/L (normal). The

From ^aAllergy Unit, Department of Dermatology, Kantonsspital; and ^bCiba-Geigy Ltd.

Reprint requests: Andreas J. Bircher, MD, Allergy Unit, Department of Dermatology, Kantonsspital, CH-4031 Basle, Switzerland.

J Allergy Clin Immunol 1996;98:994-6.

Copyright © 1996 by Mosby-Year Book, Inc.

0091-6749/96 \$5.00 + 0 1/54/76109

titer of rH-specific IgG autoantibodies was 1:1600 just before the critical injection, followed by a sharp decrease to 1:50 during the reaction, and a gradual increase to 1:3200 at 48 hours after the reaction (Fig. 1). One and 2.5 weeks later, the titer was 1:12,800, at 4 weeks 1:6400, and 18 months later still 1:1600. In three individuals previously exposed to rH, rH-specific IgG antibodies were found at 1:100 in one subject and were not detectable in the two others. Recombinant desulfato-hirudin-specific IgM antibodies were not found in any subject.

DISCUSSION

So far, rH has only rarely induced an immunologic response or reaction. In baboons, four intravenous injections of hirudin within a period of 28 days did not reveal sensitization as determined by intracutaneous tests and measurement of hirudin-specific IgG and IgM antibodies and in vitro histamine release.³ In an investigation of 263 healthy volunteers,⁴ 216 received rH subcutaneously and 47 received it intravenously. One hundred ninety-five volunteers received two single subcutaneous exposures, separated by at least 28 days, and five individuals had three exposures. Equivocal intradermal test reactions to rH (concentration 0.01 to 1.0 mg/ml) were found after one exposure (3 intravenous, 1 subcutaneous) in four individuals (1.5%), and after two exposures (1 intravenous, 1 subcutaneous) in two individuals (1%) (Table I). Positive test results after two exposures (1 intravenous, 1 subcutaneous) were present in two volunteers (1%), and after three subcutaneous exposures in one volunteer (20%) (Table I). Hirudin-specific IgG was not detected, but hirudin-specific IgE was found in three individuals, in two before administration of rH and in one after the second exposure. After the first exposure, no allergic reactions were observed. After the second or third exposure, four individuals had a reaction possibly related to rH. In three subjects (rhinitis, arthralgia, exertional dyspnea) a relationship was excluded because of negative skin test results and an unlikely temporal relationship. In one subject with local erythema and generalized pruritus after the eighth subcutaneous injection during his second exposure phase, results of intradermal tests and histamine release with rH were positive, and this reaction was judged to be related to hirudin.⁴

Recombinant desulfato-hirudin is a polypeptide, and therefore it has the potential to induce sensitization and elicit allergic reactions. Its foreign protein content from *S. cerevisiae* is less than 0.001%, as determined by an ELISA technique. Sensitization may be induced by topical application of hirudin-containing creams^{1, 2, 4} or by repeated—particularly subcutaneous—injections, as known

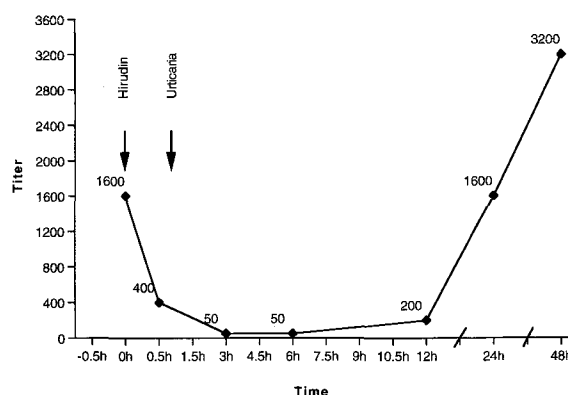


FIG. 1. Titers of hirudin-specific IgG antibodies during and after acute urticaria in response to subcutaneous recombinant hirudin.

from other heterologous peptides such as insulin and protamine.

In our patient no sensitization after the earlier five intravenous injections could be demonstrated. After his second subcutaneous injection he had an immediate-type hypersensitivity reaction. The repeated previous exposure, the time course, the clinical signs, and the positive intradermal test results at a high dilution suggest an antibody-mediated reaction. In vitro IgE antibodies to rH were not detectable. The positive skin test results could be due to specific IgG, because such skin test reactions have been related to so-called short-term sensitizing IgG antibodies.⁶ The course of the IgG antibody titer during the event also suggests an IgG-mediated anaphylactic reaction. In studies on animals³ and human beings⁴ the formation of IgG antibodies to hirudin could not be demonstrated, and IgG titers to rH in other investigations were usually not measurable or present only in very low titers according to Dr. Gippner-Steppert (personal oral communication, April 1996). Thus in our patient specific and clinically relevant IgG antibodies were present.

Tryptase measurements have been used to demonstrate mast cell degranulation.⁷ This enzyme is found in skin mast cells only and not in basophil granulocytes and is measurable in severe allergic reactions, particularly in anaphylaxis.⁷ In our patient tryptase levels were negative at several time points, indicating that either mast cells had not been activated or that this enzyme was not released in amounts sufficient to reach measurable concentrations.

We suggest that our patient may have been sensitized during his first exposure in 1989, although skin test results were negative at that time. The first subcutaneous injection 5 years later may

TABLE I. Scores of intradermal skin tests after one, two, or three exposures to hirudin

Subject No.	Exposures (no.)	Reaction		Recombinant hirudin		
		Saline	Histamine	0.01 mg/ml	0.1 mg/ml	1.0 mg/ml
5	1	0	5	1	1	1
6*	1	0	5	0	1	1
7	1	0	5	0	1	1
8	1	0	2	1	ND	ND
6*	2	0	5	4	4	4
9	2	0	2 (prick)	1	3	3
10	2	0	2 (prick)	0	1	ND
11	2	0	1	0	1	1
12	3	0	5	3	4	3

Modified from Close et al. *Coron Artery Dis* 1994;5:943-9.

Scores (0-5): 0, negative; 1, 2, suspect; 3-5, positive.

ND, Not done.

*Subject 6 had a second exposure after repeated negative test results.

have boosted IgG synthesis as shown by the increased titer just before the second subcutaneous injection. This injection resulted then in a time-dependent consumption of IgG and in clinical symptoms, possibly by the formation of immune complexes. Eighteen months after the event, IgG antibodies were still measurable in high titers, indicating that the patient is still sensitized.

The pathogenic role of IgG antibodies or their subclasses in immediate-type hypersensitivity reactions to drugs has been rarely established.⁸ However, in immediate-type hypersensitivity reactions to some drugs such as protamine,⁹ thiamine,¹⁰ and clinical dextran,¹¹ a pathogenic role of IgG antibodies has been suggested. A very similar reaction pattern, mediated by IgG antibodies, has been demonstrated in immune complex anaphylaxis to clinical dextrans.¹¹

The exact allergenic determinant of rH is not known. The solution contains only mannitol as additive in the vehicle, and results of skin tests with it were negative. A contamination with heterologous proteins during the production in *S. cerevisiae* cell cultures is possible. However, the foreign protein content is less than 0.001%, and sensitization to *S. cerevisiae* was excluded by skin and in vitro tests. Thus the allergenic determinant is probably localized at the rH molecule itself.

In summary, we report an individual who experienced an immediate-type allergic reaction after repeated exposure to rH. The results of the investigations suggest an IgG-mediated anaphylactic reaction. Because individuals with repeated but noncontinuous, subcutaneous application of hirudin may have an increased risk of sensitization, it

may be advisable to closely monitor such patients on reexposure to this compound.

We thank Dr. C. Gippner-Steppert and Prof. Hans Fritz, Abteilung für Klinische Chemie und Biochemie, Chirurgische Klinik, Munich Germany, for the determination of IgG and IgM; and Brigitta Grell, Kabi Pharmacia Diagnostics AB, Sweden, for the measurement of the specific IgE antibodies.

REFERENCES

- Dejobert Y, Martin P, Thomas P, Bergoend H. Contact dermatitis from topical leech extract. *Contact Dermatitis* 1991;24:366-7.
- Gail H, Frommer A. Kontaktallergie auf den Blutegel-Extrakt Rohhirudin. *Dermatosen* 1993;41:77-8.
- Bichler J, Gemmerli R, Fritz H. Studies for revealing a possible sensitization to hirudin after repeated intravenous injections in baboons. *Thromb Res* 1991;61:39-51.
- Close Ph, Bichler J, Kerry R, et al. Weak allergenicity of recombinant hirudin CGP 39393 ("REVASC") in immunocompetent volunteers. *Coron Artery Dis* 1994;5:943-9.
- Serruys PW, Hermann JPR, Simon R, et al. A comparison of hirudin with heparin in the prevention of restenosis after coronary angioplasty. *N Engl J Med* 1995;333:757-63.
- Brighton WD. Frequency of occurrence of IgG (S-TS). *Clin Allergy* 1980;10:97-100.
- Schwartz LB, Metcalfe DD, Miller JS, et al. Tryptase levels as an indicator of mast-cell activation in systemic anaphylaxis and mastocytosis. *N Engl J Med* 1987;316:1622-6.
- Coleman JW. Allergic reactions to drugs: current concepts and problems. *Clin Exp Allergy* 1990;20:79-85.
- Weiss ME, Nyhan D, Peng Z, et al. Association of protamine IgE and IgG antibodies with life threatening reactions to intravenous protamine. *N Engl J Med* 1989;320:886-92.
- Proebstle TM, Gall H, Jugert K, et al. Specific IgE and IgG serum antibodies to thiamine associated with anaphylactic reaction. *J Allergy Clin Immunol* 1995;95:1059-60.
- Kraft D, Hédin H, Richter W, et al. Immunoglobulin class and subclass distribution of dextran-reactive antibodies in human reactors and nonreactors to clinical dextran. *Allergy* 1982;37:481-9.