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Hypersensitivity to Nadroparin Calcium

Case Report and Review of the Literature

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The use of heparin to prevent or treat venous and pulmonary thrombosis, unstable angina and non-ST-elevation myocardial infarction is widely accepted. Nowadays, for many of these indications the unfractionated heparins (UFH; molecular weight [MW] 3–30 kDa) are largely being replaced by low-molecular-weight heparins (LMWHs; MW ≤5 kDa) because of the improved pharmacokinetic and pharmacodynamic properties as well as a better safety profile of the latter.[1-3] Adverse reactions to heparins include haemorrhages, osteopenia and immune-mediated hypersensitivity reactions, e.g. heparin-induced thrombocytopenia without and with (fatal) thrombosis,[4-10] immediate hypersensitivity reactions such as rhinoconjunctivitis,[11] asthma,[12] urticaria, angio-oedema and anaphylaxis,[12-25] and delayed cutaneous reactions generally manifesting as infiltrated, eczematous plaques or leukocytoclastic vasculitis (type III Arthus reaction) at injection sites.[26-35]

1. Case Report

A 47-year-old man with severe heart failure due to a dilated cardiomyopathy was admitted for cardiac transplantation screening. His medical history included pacemaker implantation because of a sick sinus syndrome with syncope, depression, osteopenia, gastritis, antral ulcers, a small biliary cyst and ethyl abuse, which was stopped 20 years before admission. In order to perform right and left side cardiac catheterisation, oral anticoagulants were switched to nadroparin calcium (Fraxiparine®, Sanofi-Synthelabo, Brussels, Belgium)[1] 7.500U AXa IC (Institut Choay) twice daily. Ten days after starting this preservative-free LMWH he developed a large eczematous plaque (5 × 15cm) at the injection site. There was neither thrombocytopenia nor eosinophilia. The lesion subsided within 1 week after stopping the drug and application of a topical corticosteroid.

Investigations were performed in the patient and in a healthy control who was free of any medication.

A lymphocyte transformation test (LTT) with nadroparin calcium assessed by the incorporation of [methyl-^{3}H] thymidine[36] was strongly positive with a stimulation index, i.e. counts per minute (cpm) of the nadroparin calcium-stimulated lymphocytes divided by the cpm cultured with serum-free medium, of 135 (normal <2). Flow cytometric analysis of *in vitro* nadroparin calcium-activated basophils (BAT)[37] showed a CD63 upregulation above spontaneous expression of 63% (normal <15%). Meanwhile, the patient was re-administered nadroparin calcium accidentally, which resulted in an immediate local erythematous eruption of several centimetres and a generalised pruritus and urticaria.

1 The use of trade names is for product identification purposes only and does not imply endorsement.

Table I. Summary of diagnostic investigations

	Patient	Control	Normal
Total IgE (kU/L)	16	27	<120
LTT			
nadroparin calcium	135	1.0	<2
dalteparin	1.6	ND	<2
sodium heparin	1.1	1.1	<2
BAT			
without drug	14	6	<15
anti-IgE	71	70	>15
nadroparin calcium	63	0	<15
dalteparin	0	0	<15
Sodium heparin	0	0	<15
SPT dalteparin	0/0	ND	
SPT sodium heparin	0/0	ND	
IDT dalteparin	0/0	ND	
IDT sodium heparin	0/0	ND	
SCPT dalteparin	0/0	ND	
IVPT sodium heparin	Uneventful	ND	

BAT = basophil activation test. Results shown as net % CD63-positive basophils after activation; **IDT** = intradermal test (0.05mL; serial dilution starting at 10^{-3} up to neat solution). Results shown as wheal/flare size in millimetres after 30 min; **IVPT** = intravenous provocation test; **LTT** = lymphocyte transformation test. Results expressed as stimulation index (counts/min drug/counts/min negative control); **ND** = not done; **SCPT** = subcutaneous provocation test (serial dilution starting at 10^{-3} up to neat solution); **SPT** = skin prick test (serial dilution starting at 10^{-3} up to neat solution). Results shown as wheal/flare size in millimetres after 10 min.

Forty-eight hours after administration an infiltrated plaque could be observed at the injection site.

Because the patient was scheduled for a heart transplantation, further investigations included LTT, BAT and skin testing, and subcutaneous provocation with another LMWH (dalteparin [Fragmin®, Pharmacia, Diegem, Belgium]) and intravenous provocation with unfractionated sodium heparin. All these investigations remained negative, and he underwent cardiac transplantation and was treated with unfractionated heparin uneventfully (table I).

2. Discussion

LMWHs that are derived from UFH by chemical or enzymatic hydrolysis (e.g. dalteparin, certoparin, enoxaparin, nadroparin calcium and tinzaparin) are increasingly being used in the management of venous thromboembolism and acute coronary syndromes. Furthermore, LMWHs are also intensively used in renal care (haemodialysis, haemofiltration and continuous veno-venous haemofiltration). The advantages of these drugs over UFH are largely attributable to their shorter polysaccharide chain length, which induces less nonspecific binding to cell surfaces and plasma proteins.[3] Unpredictable anticoagulant response, heparin resistance and immune heparin-induced thrombocytopenia are explained by nonspecific binding of UFH. For example, in immune heparin-induced thrombocytopenia type II, a minimum of 12–14 saccharides are required to form the antigenic complex with platelet-factor 4 and to elicit the formation of IgG antibodies against this neoantigen.[38-41] For an extensive review regarding the pathophysiology and management of heparin-induced thrombocytopenia type II, the reader is referred elsewhere.[8,42] Heparin-induced thrombocytopenia type I has only minor clinical relevance and is due to direct platelet activation by heparin.[43]

Delayed-type skin hypersensitivity (DTH) to LMWHs was first reported in 1990 in a patient who had already presented comparable lesions for UFH.[26] Induction of sensitisation may take from a few weeks to months. Once sensitised, the reaction generally occurs within 2–3 days after injection. Typically, pruritic infiltrated or eczematous plaques

occur at the site of injection; however, more generalised reactions have been described.[44] The differential diagnosis comprises haematoma, infection, contact allergy to disinfectants or preservatives, and the cutaneous manifestations of heparin-induced thrombocytopenia, which may start as erythematous plaques and precede (dramatic) skin necrosis.[26,45,46] Histopathology of the skin lesions shows spongiotic dermatitis,[26] and immunohistochemical investigations are consistent with a T-cell-mediated reaction.[26,47]

With a few exceptions, patients generally reacted to both UFH and (several) LMWHs, indicating extensive clinically relevant cross-reactivity between these drugs.[28,30-32,44,47-54]

DTH to LMWH has been reported to be more prevalent in obese and diabetic women,[30,32] leading to the presumption that hormonal factors and/or longer deposition related to the skin-fold thickness might facilitate sensitisation.[55]

Heparin salts can elicit DTH reactions but also, albeit on very rare occasions, immediate hypersensitivity reactions such as rhinoconjunctivitis,[11] asthma,[12] urticaria, angio-oedema and generalised anaphylaxis.[12-25] It is not known whether pre-existing DTH to heparins predisposes to other immune-mediated adverse reactions such as immediate hypersensitivity or heparin-induced thrombocytopenia type II. Considering our case history and the occurrence of heparin-platelet factor 4 antibodies with or without thrombocytopenia or thrombosis,[26,40,51] one should always be alert to this possibility. Subcutaneous challenge with heparins is now accepted as the most reliable method to identify DTH reactions to heparins, whereas patch tests yield a high rate of false-negative results.[26,30-32,50,54,56] To avoid false-negative reactions it is important to perform a delayed reading after 72–96 hours.[53] In addition, the technique is recommended to determine safe treatment options for patients allergic for specific heparins.

In accordance with other investigators,[30,35,49] we believe that, for safety reasons, *in vitro* tests and appropriate skin tests should precede subcutaneous challenge tests. Therefore, as an initial step we performed *in vitro* lymphocyte transformation and basophil activation tests. The LTT, i.e. quantification of antigen-specific lymphocyte proliferation responses, is classically considered to mirror cellular immunity and has already been described to be useful in the evaluation of heparin-induced DTH.[30] In our patient, the LLT was strongly positive for nadroparin calcium, but remained negative for dalteparin and UFH.

Basophils activated with specific allergen that is recognised by surface receptor FcεRI-bound IgE not only secrete and generate quantifiable bioactive mediators, but also upregulate the expression of certain markers that can be detected by multicolour flow cytometry.[57] CD63 (gp53) is a member of the transmembrane-4 superfamily, and can be detected in a wide variety of cell types, e.g. leucocytes and platelets.[58,59] In resting basophils, CD63 is anchored in the basophilic granule membrane and is only weakly expressed on the surface membrane.[60] In contrast, as a result of fusion between the granule and plasma membrane, CD63 is expressed with a high density on activated basophils, and mirrors histamine release.[61] As shown in table I, nadroparin calcium induced substantial activation of the basophils from the patient, with an upregulation of CD63 expression from 14% to 63%. In contrast, expression of CD63 on the basophils from the control subject remained unaltered. In addition, the BAT was negative for dalteparin and UFH for both the patient and the control individual. The BAT does not allow discrimination between an anaphylactic or an anaphylactoid reaction; however, this is not a drawback in the investigation of such drug-induced reactions. Some drugs act as nonspecific histamine releasers and in other cases, due to the physicochemical characteristics of the drug or its excipients, it might be impossible to couple the drug to a solid phase and to determine specific IgE.[62] Moreover, analysis of CD63 upregulation on passively sensitised basophils, i.e. donor basophils that were incubated with serum of the patient, has been shown to suggest the presence of drug-specific IgE antibodies.[63] The patient's clinical manifestations during initial treatment and after inadvertent re-

administration and the positive LTT and BAT test strongly suggest the combined presence of a DTH and immediate-type hypersensitivity to nadroparin calcium. Moreover, the negative *in vitro* tests, skin tests and finally the challenge test that was used as an *ultima ratio* measure, proved useful to tailor the therapeutic alternative.

In 1994, Patriarca et al.[21] first reported a successful hyposensitisation in a woman with urticaria to heparin who needed extracorporeal circulation for a mitral valve replacement. Some anecdotal reports have shown that hyposensitisation with heparin might be effective in immediate-type hypersensitivity to heparins.[22] No controlled studies have been performed to support this therapeutic option.

Heparinoids such as danaparoid that mainly consist of heparan sulfate cannot be recommended as a substitute for UFH or LMWH.[35,52,64,65] Obviously, danaparoid should be included in the testing programme of patients, in particular those who are intolerant for multiple LMWH.[22]

Hirudins are highly homologous polypeptides from the medicinal blood-sucking leeches (*Hirudo medicinalis*). They have a high and specific affinity for thrombin and are consequently potent anticoagulants. Recently, recombinant hirudins (r-hirudins) such as desirudin and lepirudin have become available for therapeutic use.[66,67] Cross-reactivity between hirudins and heparins is unlikely since no common epitopes have been found.[68] Therefore, r-hirudin or its analogues can be recommended as an alternative for patients with immune-mediated reactions to heparin preparations including danaparoid.[54,65,69] It should be noted that antihirudin antibodies and allergic reactions, including urticaria, DTH and Arthus reactions, have been reported.[69-73]

3. Conclusion

We presented a case of combined immediate (BAT) and delayed (LTT) hypersensitivity reactions to nadroparin calcium in a patient tolerant for UFH and dalteparin. Because inadvertent re-administration of nadroparin calcium resulted in an immediate local reaction, generalised pruritus and urticaria as well as a delayed infiltrated plaque at the injection site, skin tests with nadroparin calcium were not performed. The *in vitro* assays combined with appropriate skin and challenge tests allowed us to establish a safe alternative anticoagulant regimen for our patient. Comprehensive studies are required to further validate our results and allow the introduction of these *in vitro* tests in mainstream diagnostic use.

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