

Anaphylaxis after intra-articular injection of orgotein

Detection of an IgE-mediated mechanism

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Orgotein, a recently introduced anti-inflammatory agent, is the generic name accepted in the U.S.A. for the drug version of copper-zinc superoxide dismutase. This metallo-enzyme is obtained from bovine liver and has a molecular weight of about 32,000. We report on a patient to whom an intra-articular injection of orgotein caused an anaphylactic reaction. An IgE-mediated mechanism was suggested by an immediate positive skin test to orgotein and by the finding of specific IgE against orgotein in the patient's serum.

Key words: anaphylaxis; drug hypersensitivity; superoxide dismutase.

Accepted for publication 6 September 1986

Orgotein is the generic name accepted in the U.S.A. for the drug version of Cu Zn superoxide dismutase. It occurs naturally in all mammalian cells and is obtained from bovine liver by a process involving heat treatment, enzymatic digestion, and purification by chromatography.

Preliminary investigations of orgotein have shown that it is an extremely powerful superoxide (O_2^-) dismutase. This enzyme dismutates superoxide anions (O_2^-) formed by a host of intracellular autoxidations into O_2 and H_2O_2 , thus protecting the interior of the cell against the toxic effects of O_2 and its derivatives (6). The orgotein has a molecular weight of approximately 32,000 and its toxicologic, pharmacologic and therapeutic properties have been reviewed (3, 4) but clinical experience with this drug is still very limited.

Orgotein has anti-inflammatory properties and it has been given by intra-articular injections in degenerative joint disease (2, 5) and for the amelioration of side effects of radiation therapy (7).

Orgotein for injection is presented as a freeze-dried solid with sucrose as excipient (2 mg/1 mg protein) and sodium chloride pH 6.5-7 as diluent. Solubility is 150 mg/ml and the shelf life 5 years at room temperature.

This anti-inflammatory agent has recently been introduced for human use in some European countries and the only side effects described to date are local, painful subcutaneous reactions, sometimes with erythema and pruritus, and urticaria (1).

The following case report is of a patient who developed generalised urticaria, severe angio-oedema and hypotension after an intra-articular injection of this drug.

CASE REPORT

A 33-year-old man with arthritis of his right knee was treated weekly with intra-articular injections of 4 mg orgotein (Peroxinorm, Andrómaco, S.A., Lab., Madrid). The vial containing lyophilised orgotein was reconstituted with 2 ml saline solution before administration. These injections were given together with mepivacaine hydrochloride (Scandicain 3% Inibsa, S.A., Lab., Barcelona). Three months after the beginning of the treatment and, about 15 min after an intra-articular injection, he developed generalised urticaria and severe angio-oedema of his face, tongue and genitals. In the emergency room he was treated with epinephrine, antihistamines

and corticosteroids. The oedema disappeared over the next few hours and the patient was discharged. After the above reaction, the orgotein treatment was discontinued; but, 2 months later, a new intra-articular injection of orgotein (without mepivacaine this time because it was considered responsible for the previous reaction) was given. Within 1 min, the patient noticed generalised pruritus and tongue swelling. Shortly afterwards, he developed dysnea, cyanosis and hypotension (BP:70/30 mmHg). He required admission to an intensive care unit and administration of epinephrine, intravenous fluids, antihistamines and hydrocortisone. Recovery from this reaction was obtained over the next few hours, enabling the patient to be discharged within 36 h. The patient had no other symptoms of protein allergy of bovine origin and no other allergic diseases.

METHODS

Skin tests. Six weeks later, skin tests with orgotein were performed as follows: 4 mg lyophilised orgotein were diluted in 40 ml of 0.9 % saline solution. After stirring for 15 min, the solution was passed through a Millipore filter for sterilization and 10-fold dilutions were prepared. Prick and intradermal tests with orgotein dilutions, starting with a concentration of 10^{-6} mg/ml were done. A battery of commercially available common inhalants (Dôme-Hollister-Stier) and both, positive (histamine) and negative (saline) skin test controls were also tested (10). The results were read after 15 min by measuring the diameter of the wheal.

The same tests were performed on five atopic subjects with seasonal rhinitis and/or asthma.

In addition, to rule out an unlikely participation of mepivacaine at the first reaction, we also performed a skin test and subcutaneous challenge (up to 1 ml) with 3 % mepivacaine on our patient (11).

Reverse enzyme immunoassay (REIA) for determination of specific IgE antibodies against orgotein. Two serum samples obtained from the patient, 24 h (serum 1) and 6 weeks (serum 2), respectively, after the last reaction, were tested for specific IgE.

Allergen-labelling procedure. The antigen was conjugated with peroxidase as follows: 0.5 mg orgotein

diluted in 125 μ l phosphate-buffered saline (PBS) was mixed with 1 mg oxidated peroxidase and 200 μ l carbonate-bicarbonate buffer 1 M pH 9.5. After 18 h at 4°C, 1 ml H₂O containing 1 mg sodium borohydride was added. Finally, this conjugate solution was dialysed for 24 h against PBS-25 % glycerol.

REIA for IgE against orgotein. The assay uses M24AR microplates (Dynatech Co., Alexandria, Va.) as a solid phase. These microplates were previously optimally coated with a monospecific anti-human IgE (Tago Inc., Burlingame, CA) as reported (8, 9). The different sera (0.05 ml) mixed with 0.05 ml PBS-0.1 % Tween 20-25 % fetal calf serum (PBS-Tw-FCS) were incubated in triplicate wells for 18 h at 4°C while shaking. After several washings in PBS-Tw and tap water, 0.5 μ g of peroxidase conjugate orgotein in 0.2 ml PBS-Tw-FCS was added to each well and left for 1 h while shaking. Later, the washing steps were repeated before adding 0.1 ml of the substrate o-phenylenediamine-H₂O₂ per well (8, 9). After 30 min the reaction was arrested by adding 0.1 ml of 2N H₂SO₄. The plates were read at 492 nm on a Titertek Multiskan (Flow Laboratories, Irvine, Scotland). Any result higher than the control values plus 2 SD was considered positive.

Specificity controls. Serum 2 of the patient (obtained 6 weeks after the last reaction) was incubated for 18 h at 4°C as described above. After washing, the peroxidase labelled orgotein (0.5 μ g/well) was added together with 20 μ g of the unlabelled orgotein or 20 μ g of three unrelated antigens (trypsin, *Lolium perenne*, *Dermatophagoides pteronyssinus*).

RESULTS

Skin tests. An immediate positive response (6 $\times$ 6 mm wheal) was obtained in the orgotein prick test with a concentration of 0.1 mg/ml. A positive response (19 $\times$ 16 mm wheal with pseudopods) was obtained after intradermal test at 0.001 mg/ml. These responses were larger than those obtained with the histamine controls. Skin tests

with common inhalants and saline control were negative.

Five atopic subjects did not react after prick and intradermal test with orgotein at a concentration of 0.1 mg/ml.

Skin test and subcutaneous challenge with mepivacaine performed in our patient were negative.

REIA. A significant level of specific IgE was found in the patient's sera. As seen in Table 1, the results obtained with serum 2 were higher than those obtained with serum 1 and both higher than the controls. In addition, the orgotein inhibited the reaction, whereas there was no inhibition with the three unrelated antigens. Thus, the REIA inhibition study confirmed the specificity of the assay for orgotein.

Comment

Orgotein has recently been introduced for human use in some European countries and is employed as an anti-inflammatory agent (2, 5, 7). We have found no references in the literature about anaphylactic reactions with this protein.

This case report should be of particular interest because: 1) the induction of anaphylactic reactions after the injection of orgotein on two separate occasions suggested that such reactions were precipitated by this drug, and 2) an IgE-mediated mechanism was demonstrated by an immediate positive skin test to orgotein and by the

finding of specific IgE antibodies to orgotein by REIA.

REFERENCES

1. Del Favero, A.: Anti-inflammatory analgesic and drugs used in rheumatoid arthritis and gout. In Dukes, M. N. C. (ed.): Side effects of drugs, pp. 100. Vol. 6. Excerpta Medica, Amsterdam 1982.

2. Goebel, K. M., Storck, U. & Neurath, F.: Intrasinovial orgotein therapy in rheumatoid arthritis. *Lancet* 2, 1015-1017, 1981.

3. Hassan, H. M. & Fridovich, I.: Chemistry and biochemistry of superoxide dismutase. *Eur. J. Rheumatol. Inflamm.* 4, 160-172, 1981.

4. Huber, W. & Menander-Huber, K. B.: Orgotein. *Clin. Rheum. Dis.* 6, 465-476, 1980.

5. Lund-Olesen, K. & Menander-Huber, K. B.: Orgotein: a new anti-inflammatory metalloprotein drug. Preliminary evaluation of clinical efficacy and safety in degenerative joint disease. *Curr. Ther. Res.* 16, 706-717, 1974.

6. McCord, J. M. & Fridovich, I.: Superoxide-dismutase, an enzymatic function for erythrocuprein. *J. Biol. Chem.* 244, 6049-6055, 1969.

7. Menander-Huber, K. B., Edsmyr, F. & Huber, W.: Orgotein therapy. *Scand. J. Urol. Nephrol. (Suppl)* 55, 219-224, 1980.

8. Moneo, I., Cuevas, M., Ureña, V., Alcover, R., Bootello, A.: Reverse enzyme immunoassay for the determination of *Dermatophagoides pteronyssinus* IgE antibodies. *Int. Arch. Allergy Appl. Immunol.* 71, 285-287, 1983.

9. Moneo, I., Ureña, V., Dominguez, J., Bootello, A.: Reverse enzyme immunoassay for the determination of *Lolium perenne* IgE antibodies. *Int. Arch. Allergy Appl. Immunol.* 72, 184-187, 1983.

10. Norman, P. S.: In vivo methods of study of allergy. In Middleton, E., Reed, C. E. & Ellis, E. F. (eds.): *Allergy. Principles and practice*, pp. 295-302, C. V. Mosby, St. Louis, 1983.

11. Shatz, M.: Skin tests and incremental challenge in the evaluation of adverse reactions to local anesthetics. *J. Allergy Clin. Immunol.* 74, 606-616, 1984.

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Table 1

Determination of specific IgE to orgotein by REIA	
Serum	OD ₄₉₂ *
Blank	0.040±0.015
7 control sera	0.052 ±0.012
Serum 1	0.205±0.015
Serum 2	1.010±0.020
REIA inhibition assay	
Serum 2 + 20 µg trypsin	1.040±0.030
Serum 2 + 20 µg <i>Lolium perenne</i>	1.050±0.040
Serum 2 + 20 µg <i>D. pteronyssinus</i>	1.010±0.030
Serum 2 + 20 µg unlabelled orgotein	0.125 ±0.015

* OD: Optical densities
Plates were read at 492 nm on a Titertek Multiskan

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