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Delayed hypersensitivity to bosentan

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Key words: bosentan; delayed hypersensitivity; drug rash (or reaction) with eosinophilia and systemic symptoms; lymphocyte transformation test.

Bosentan is a competitive antagonist of endothelin (ET)-1 at the ET-A and ET-B receptors and is indicated for the treatment of pulmonary hypertension. Bosentan is generally well tolerated; nevertheless, a recent study concerning the European post-marketing surveillance of bosentan (1) has reported a 7.6% incidence of serum aminotransferase elevation in 4623 patients under treatment. To our knowledge, however, only one case of severe liver dysfunction associated with bosentan therapy has been reported. It was characterized by a very high level of serum aminotransferase, which developed in the initial 4 to 6 months of treatment, and was reversible without sequelae either during continued treatment with bosentan or upon the discontinuation of therapy (2).

This is the first case of a cell-mediated hypersensitivity to bosentan diagnosed on the basis of positive responses to the lymphocyte transformation test and a challenge. However, the latter provoked a severe reaction (DRESS).

We studied a 44-year-old Hispanic woman who had experienced a generalized maculopapular exanthema (MPE) on the 18th day of therapy with bosentan for pulmonary hypertension caused by her scleroderma. Her symptoms subsided after 10 days of therapy with betamethasone orally (4 mg/day on the first 3 days, then 1 mg/day). Her personal history was negative for allergic diseases. One month after the resolution of the symptoms, she underwent diagnostic tests. On the first day, we performed skin tests, patch tests, and a lymphocyte transformation test (LTT). For skin testing, we used concentrations of 0.2, 2.0, and 20 mg/ml 0.9% NaCl. We prepared bosentan sterile solutions no more than 2 h before testing, using the powder obtained from a tablet, as previously described (3). We initially tested the reagent solutions on volar forearm skin by the prick method and, in case of negative results, we injected 0.02 ml of the solutions intradermally. Readings were made 20 min and 48 h after the injections. For patch testing, we used bosentan at a concentration of 200 mg/ml 0.9% NaCl, prepared as above. The reading was made after 48 h. The LTT was performed according to the Nyfeler and Pichler method (4), adding bosentan to the medium at concentrations ranging from 1 to 100 μg/ml (Table 1). Tetanus toxoid (Instituto Berna, Barcelona, Spain) was used as a control antigen. The stimulation index (SI) was calculated as counts per minute of antigen-containing cultures divided by counts per minute of control cultures (without antigen). A SI higher than 2.0 was considered a positive result. We also performed all the above tests in three subjects with scleroderma who had previously tolerated bosentan.

Delayed-reading skin tests and patch tests were negative in both the patient and the controls. The LTT was positive only in our patient and at concentrations of 10 μg (SI = 2.1) and 100 μg (SI = 3.8), as shown in Table 1.

Considering that the drug was so important in the patientís case, that the results of *in vivo* and *in vitro* tests were not consistent, and that the patient had not suffered a severe reaction, and in order to establish a firm diagnosis, we performed a single-blind, placebo-controlled oral provocation test (OPT), according to the position paper by the European Network for Drug Allergy (ENDA) (5). On the first day, we administered a placebo (talc) and no adverse reaction occurred. After 1 week, we administered one hundredth (0.625 mg) of the bosentan therapeutic dose; 24 h later the patient experienced generalized pruritus, trunk erythema, facial angioedema, fever, rash, and coughing, with a decrease of more than 34% in the forced expiratory volume at 1 s (FEV_1), as well as eosinophilia, lymphopenia, hyperbilirubinemia, increased liver enzyme and creatinine levels, and renal colic. We made a diagnosis of drug rash (or reaction) with eosinophilia and systemic symptoms (DRESS), according to the new RegiSCAR group criteria (6). Table 1 shows the blood values at baseline, at the first symptom onset, and remission. The patient was immediately treated with 10 mg of chlorpheniramine and 40 mg of methylprednisolone intravenously, as well as 2 puffs of 100 μg salbutamol spray and oxygen (5 l/min). The fever and rash completely resolved within 17 days, with normalization of the creatinine level, eosinophilia count, and FEV_1. The patient continued the treatment with the first two drugs for 15 days, with complete resolution of the symptoms.

To our knowledge, this is the first case of a cell-mediated hypersensitivity to bosentan.

In our patient, the LTT positivity was confirmed by the OPT result. Thus, the LTT proved to be more sensitive than delayed-reading skin tests and patch tests, enabling us to diagnose a cell-mediated hypersensitivity to bosentan. This higher sensitivity of the LTT has been observed in other studies concerning non immediate reactions to β-lactams (7) and other drugs, such as non β-lactam antibiotics and anticonvulsants (8). On the other

Table 1. Results of blood values, lymphocyte transformation test (LTT)*, and forced expiratory volume at 1 s (FEV_1)**

	Baseline	First symptom onset	Day 5 after reaction	Day 10 after reaction	Day 17 after reaction	Day 32 after reaction (remission)	SI
RBC ($\times 10^6$/μl)	4.70	4.57	4.26	4.40	4.60	4.51	
Hb (g/dl)	13.8	13.8	12.7	13.9	13.9	13.2	
WBC ($\times 10^3$/μl)	4.70	8.20	6.45	5.14	5.53	5.28	
Neutrophils ($\times 10^3$/μl) (%)	2.63 (56%)	1.79 (39.2%)	3.20 (49%)	2.80 (54.5%)	2.89 (52.2%)	3.13 (59.3%)	
Lymphocytes ($\times 10^3$/μl) (%)	1.28 (27.3%)	0.72 (15.9%)	1.14 (17.5%)	1.40 (27.2%)	1.53 (27.6%)	1.07 (20.2%)	
Monocytes ($\times 10^3$/μl) (%)	0.39 (8.2%)	0.25 (5.8%)	0.41 (6.2%)	0.36 (6.9%)	0.45 (8.1%)	0.37 (7%)	
Eosinophils ($\times 10^3$/μl) (%)	0.27 (5.7%)	3.01 (36.8%)	1.56 (23.9%)	0.37 (7.2%)	0.48 (8.7%)	0.53 (10.1%)	
Basophils ($\times 10^3$/μl) (%)	0.06 (1.2%)	0.10 (2.3%)	0.14 (2.1%)	0.10 (1.9%)	0.09 (1.7%)	0.06 (1.2%)	
PLT ($\times 10^3$/μl)	296	316	343	399	384	387	
AST (U/l)	17	256	299	108	21	21	
ALT (U/l)	16	576	748	442	75	26	
γGT (U/l)			593	489	247	61	
Total bilirubin (mg/dl)			2.29	1.52	1.13	0.48	
Direct bilirubin (mg/dl)			0.86	0.49	0.37	1.03	
FEV_1% predicted		34%**	88%				
LTT at 100 μg concentration							3.8
LTT at 10 μg concentration							2.1
LTT at 1 μg concentration							1.4

*LTT was performed 1 month after the maculopapular exanthema resolution.
**FEV_1% predicted performed 2 h after the reaction was 60%.

hand, the activation of drug-specific T cells plays a central role in many non immediate drug hypersensitivity reactions, such as MPE, toxic epidermal necrolysis (TEN), DRESS, and acute generalized exanthematous pustolosis (AGEP) (8, 9). Therefore, a more extensive diagnostic use of the LTT – which is still generally considered merely a research tool – could improve the sensitivity of the diagnostic workup for non immediate reactions to drugs, reducing the number of drug provocation tests, which are not only harmful, but are also contraindicated in severe reactions such as TEN, DRESS, and AGEP. However, a recent study by Kano et al. (9) proved that the LTT sensitivity varies depending on the type of drug reaction and the time interval between such reactions and testing. In fact, positive results were obtained when the test was performed at the acute stage, but not at the recovery stage, in MPE and TEN, while positive responses were observed at the recovery stage, but not at the acute one, in DRESS, as in our case.

All authors have no relationships to declare.

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References

1. Humbert M, Segal ES, Kiely DG, Carlsen J, Schwierin B, Hoeper MM. Results of European post-marketing surveillance of bosentan in pulmonary hypertension. Eur Respir J 2007;**30**:338–344.
2. Nagai Y, Okada E, Mihara S, Sato K, Ohi S, Ishikawa O. Severe liver dysfunction due to bosentan in a patient with mixed connective tissue disease. Eur J Dermatol 2008;**18**:190–191.
3. Romano A, Guéant-Rodriguez RM, Viola M, Amoghly F, Gaeta F, Nicolas JP et al. Diagnosing immediate reactions to cephalosporins. Clin Exp Allergy 2005;**35**:1234–1242.
4. Nyfeler B, Pichler WJ. The lymphocyte transformation test for the diagnosis of drug allergy: sensitivity and specificity. Clin Exp Allergy 1997;**27**:175–181.
5. Aberer W, Bircher A, Romano A, Blanca M, Campi P, Fernandez J et al. Drug provocation testing in the diagnosis of drug hypersensitivity reactions: general considerations. Allergy 2003;**58**:854–863.
6. Kardaun SH, Sidoroff A, Valeyrie-Allanore L, Halevy S, Davidovici BB, Mockenhaupt M et al. Variability in the clinical pattern of cutaneous side-effects of drugs with systemic symptoms: does a DRESS syndrome really exist? Br J Dermatol 2007;**156**:609–611.
7. Luque I, Leyva L, José Torres M, Rosal M, Mayorga C, Segura JM et al. In vitro T-cell responses to beta-lactam drugs in immediate and nonimmediate allergic reactions. Allergy 2001;**56**:611–618.
8. Pichler WJ, Tilch J. The lymphocyte transformation test in the diagnosis of drug hypersensitivity. Allergy 2004;**59**:809–820.
9. Kano Y, Hirahara K, Mitsuyama Y, Takahashi R, Shiohara T. Utility of the lymphocyte transformation test in the diagnosis of drug sensitivity: dependence on its timing and the type of drug eruption. Allergy 2007;**62**:1439–1444.