



Contents lists available at ScienceDirect

Allergy International

journal homepage: <http://www.elsevier.com/locate/alit>

Letter to the Editor

Hypereosinophilia with rash to dobutamine infusion; sulfite hypersensitivity diagnosed by *in vitro* stimulation assays

Dear Editor,

Introduction

Dobutamine is a widely prescribed form of inotropic support for patients with heart failure, but infusion thereof has been associated with hypersensitivity reactions, namely eosinophilia or eosinophilic myocarditis.^{1–5} However, it remains unclear whether the true culprit is dobutamine per se or its excipient, sulfite.^{6,7} We report a case of recurrent hypereosinophilia with a skin rash that developed during dobutamine infusion. We used an *in vitro* flow cytometric assay to show that the hypersensitivity was attributable to sulfite.

Case

A 74-year-old male presented to the allergy clinic with hypereosinophilia (2955/mm³) and a generalized morbiliform rash. He had

been hospitalized for aggravated heart failure 2 weeks prior, and had been under supportive management that included dobutamine infusion (Fig. 1). He had multiple comorbid illnesses, including diabetes mellitus, three-vessel disease, and chronic obstructive pulmonary disease. His initial problems on admission, including dyspnea and edema, gradually improved with supportive management. However, his blood eosinophil count began to increase on hospital day (HD) #5, and a rash developed on HD#12. No fever was evident. Laboratory markers of liver and kidney function were normal. The patient's total serum IgE level was 198 IU/mL. He did not have elevated IgG antibodies against common parasites.

We reviewed his prior medical records and found that he had experienced two similar episodes of eosinophilia and/or a rash during two recent hospitalizations necessitated by aggravated heart failure, but no eosinophilia was evident during outpatient follow-ups (Fig. 1). The first episode occurred 2 years prior; his blood eosinophil count increased from 87/mm³ on HD#1 to 821/mm³ on HD#16. He received dobutamine infusions during that

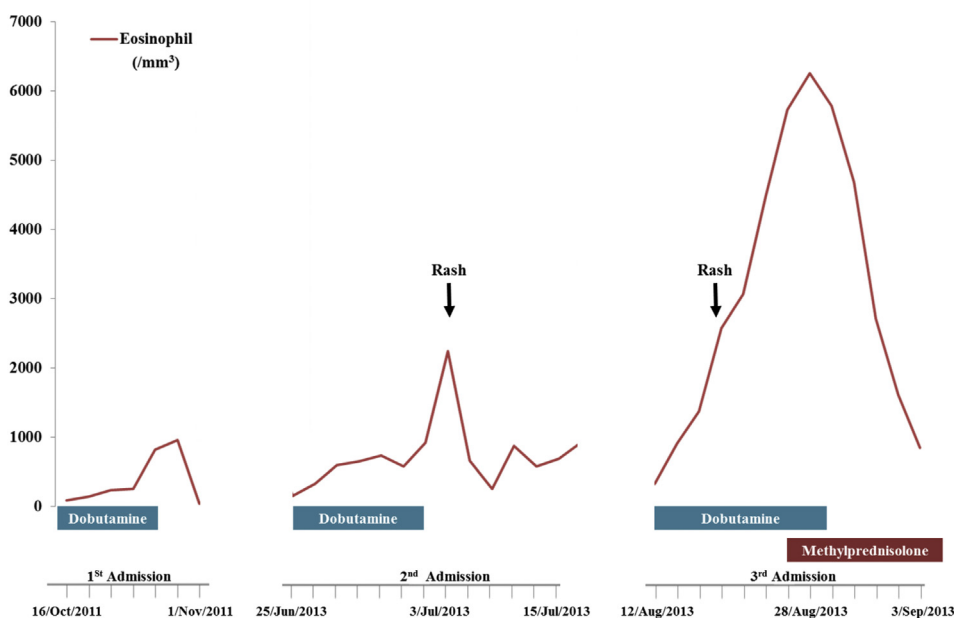


Fig. 1. Clinical courses of blood eosinophil counts, rash, and dobutamine exposure.

Peer review under responsibility of Japanese Society of Allergy.

<http://dx.doi.org/10.1016/j.alit.2016.01.008>

1323–8930/Copyright © 2016, Japanese Society of Allergy. Production and hosting by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

time. No rash had been noted. The second episode developed 3 months prior, where his blood eosinophil count increased from $36/\text{mm}^3$ on HD#1 to $2132/\text{mm}^3$ on HD#7, and a generalized morbiliform rash developed on HD#9. Dobutamine infusion was stopped on HD#8, and the eosinophilia and rash gradually

improved (eosinophil count was $572/\text{mm}^3$ on discharge day, HD#18).

Based on this history, we suspected that his current hypereosinophilia and rash were attributable to dobutamine hypersensitivity. We stopped the dobutamine infusion and prescribed a

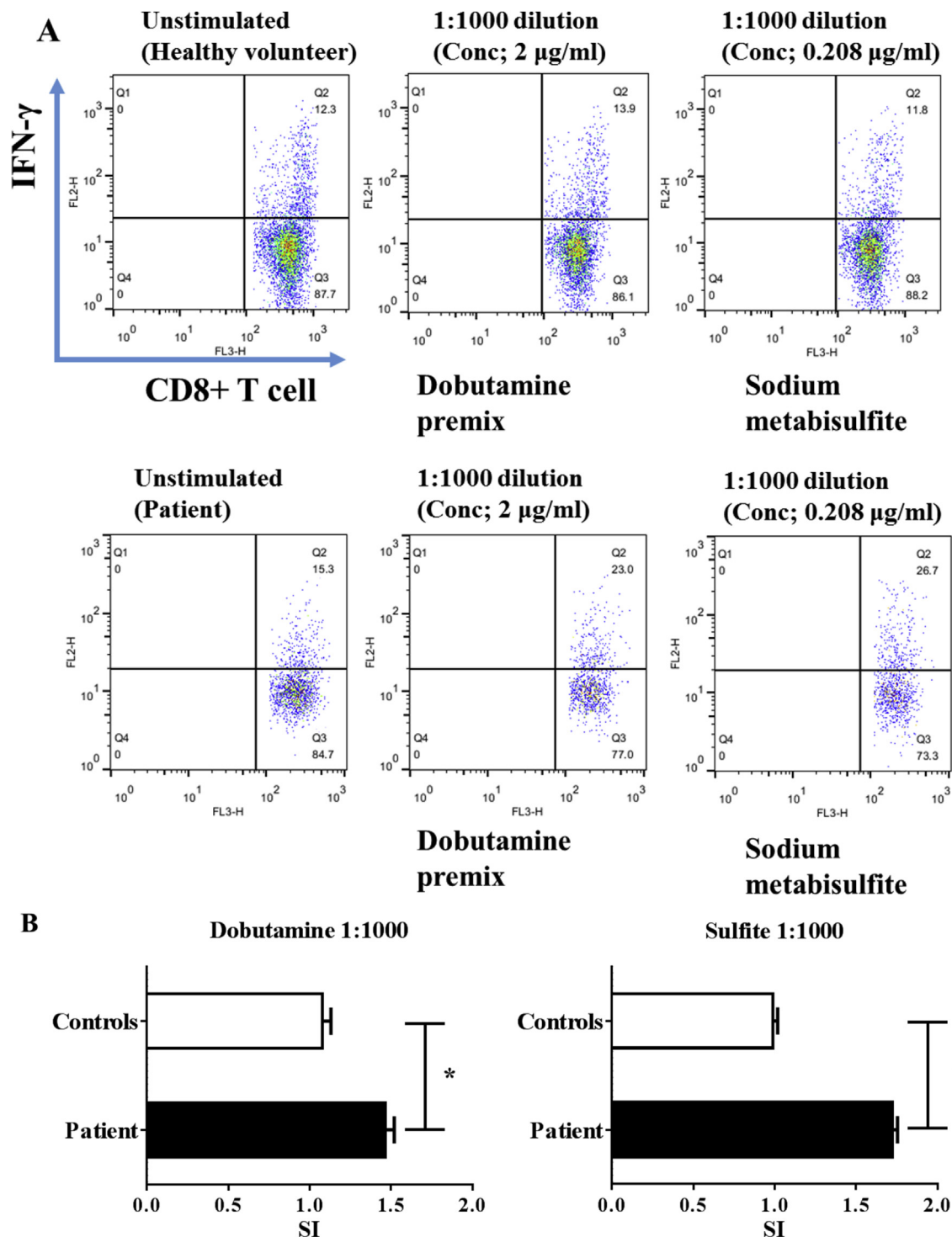


Fig. 2. *In vitro* flow cytometric measurements of interferon- γ expression in CD8+ T lymphocytes after dobutamine or sodium metabisulfite stimulation. **(A)** A representative example showing our flow cytometric measurement in CD8+ T lymphocytes were measured after 72 hours of *in vitro* stimulation with diluted dobutamine pre-mix (mid-column) or sodium metabisulfite (right column), respectively. **(B)** Bar graphs showing stimulation index (SI), which indicates the fold increase in interferon- γ expression in *in vitro* cultures with the drug stimulation compared to cultures without the stimulation. Data are expressed as mean $\pm$ standard errors of means. The difference in SI between three healthy volunteers and the patient was statistically significant (* $p < 0.05$ by the Mann-Whitney U test). Conc, concentration; SI, stimulation index.

systemic corticosteroid (methylprednisolone, 20 mg per day). The rash and eosinophil count gradually improved (eosinophil count fell from a peak of 6154/mm³ on HD#17 to 746/mm³ on HD#23); he was discharged on HD#23.

To confirm that dobutamine had caused the problems described above, we performed an *in vitro* cell stimulation assay. We obtained peripheral blood mononuclear cells (PBMCs) 4 weeks after discontinuation of systemic corticosteroid therapy. We used flow cytometry to measure the *in vitro* interferon- γ (IFN- γ) expression levels in T lymphocytes; the method was a slight modification of that reported earlier.⁸ We first tested the injectable form of dobutamine (the agent administered during development of hypersensitivity; Dobutamine HCl Pre-mix Injection; 500 mg dobutamine and 52 mg sodium metabisulfite in 5% [w/v] dextrose; 250 mL). We also examined the *in vitro* response to sodium metabisulfite (Sigma), because earlier expert reports suggested that sulfite hypersensitivity might be in play during dobutamine-associated eosinophilia or eosinophilic myocarditis.^{1,2,9} Briefly, PBMCs were isolated from whole blood by centrifugation through Ficoll-Paque-Plus solution (GE Healthcare). The cells were suspended to a concentration of 5×10^6 /mL in RPMI 1640 medium supplemented with 10% (v/v) heat-inactivated fetal bovine serum. Next, 0.1 mL amounts of cell suspension were added to the wells of a 96-well tissue culture plate. Diluted dobutamine pre-mix (in 0.1 mL culture medium) or sodium metabisulfite (also in 0.1 mL culture medium) was added to the wells. The final concentrations were determined as 2 μ g/mL (1:1000 dilution) for dobutamine, and 0.208 μ g/mL (1:1000 dilution) for sodium metabisulfite, respectively; cytotoxic effects were observed at concentrations higher than 2.08 μ g/mL (1:100 dilution) for sodium metabisulfite. The plates were incubated for 3 days (37 °C, 5% [v/v] CO₂, 100% humidity).

For each drug stimulation, IFN- γ expression levels in CD8+ T lymphocytes were measured by flow cytometry (Fig. 2A). Stimulation index (SI) was calculated as 'IFN- γ expression levels with drug stimulation divided by the levels at baseline'. All measurements were done in triplicate. IFN- γ expression levels increased compared to the baseline when CD8+ T lymphocytes were stimulated with the dobutamine pre-mix (SI 1.5). However, IFN- γ expression levels also increased with sodium metabisulfite stimulation (SI 1.7). Such responses were not observed in three healthy volunteers. The difference in SI between the patient and healthy volunteers was statistically significant (Fig. 2B). Thus, we diagnosed our patient with sulfite hypersensitivity.

Sulfite is commonly found in drugs and food products, and has been suggested to be an unsuspected cause of drug or food hypersensitivity.¹⁰ As suggested in earlier cardiology articles, sulfite has also been suspected to trigger hypersensitivity reactions in patients receiving dobutamine infusions.^{1,2,9} However, this possibility has never been directly tested. A recent case report on a patient with eosinophilic endomyocarditis showed that the dobutamine preparation used (which included sulfite) stimulated lymphocytes, but did not separately explore any possible effect of sulfite.¹¹ Our present finding suggests that it is important to determine the proportion of patients who develop sulfite hypersensitivity when infused with dobutamine preparations.

It is unclear whether our patient developed eosinophilic myocarditis caused by sulfite hypersensitivity. However, as his heart failure symptoms improved during dobutamine infusion and supportive management, we speculate that eosinophilic myocarditis was probably not in play. We observed no other relevant systemic sign or symptom, and thus concluded that the sulfite hypersensitivity, presenting with hypereosinophilia and a skin rash, did not meet the criteria for DRESS syndrome.

In vitro assays yield evidence of (only) "immunological sensitization"; such tests cannot prove that sulfite hypersensitivity actually

triggers eosinophilia and/or rash. However, the recurrent episodes of hypersensitivity developing upon dobutamine infusion and the positive *in vitro* response to sulfite strongly support a diagnosis of sulfite hypersensitivity in the present case.

We measured IFN- γ levels as the marker for acute phase reaction of CD8+ T lymphocytes to drug stimulation. In the literature, *in vitro* IFN- γ assays have been frequently used as the rapid and relatively convenient diagnostic tools to determine T-cell mediated sensitization to drugs in patients with delayed-type drug hypersensitivity.^{12,13} However, we agree that further assays for eosinophilopoietic cytokines, such as IL-5, should be necessary to detect the intrinsic nature of this clinical syndrome. Also, accumulation of cases is needed to clarify the mechanism of sulfite hypersensitivity.

In conclusion, this is the first case report on a patient exhibiting dobutamine infusion-related hypersensitivity to include *in vitro* immunological assessment of sulfite hypersensitivity. Clinical allergists need to be aware that dobutamine infusion may trigger hypersensitivity reactions (eosinophilia and/or rash). Also, the proportion of patients who are in fact hypersensitive to sulfite, should be explored.

Acknowledgements

The present study was supported by a grant no. 04-2012-0980 (2012-1336) from the SNUH Research Fund.

Conflict of interest

The authors have no conflict of interest to declare.

Sung-Yoon Kang ^{a,b,e}, Ji-Won Lee ^{b,e}, Da-Eun Park ^b, Bo-ram Bae ^b, Hyun-Seung Lee ^b, Byung-Keun Kim ^{a,b}, Ju-Young Kim ^{a,b}, Kyung-Hwan Lim ^c, Heung-Woo Park ^{a,b}, Bon-Kwon Koo ^{a,d}, Sang-Heon Cho ^{a,b}, Woo-Jung Song ^{a,b,*}

^a Department of Internal Medicine, Seoul National University College of Medicine, Seoul, South Korea

^b Institute of Allergy and Clinical Immunology, Seoul National University Medical Research Center, Seoul National University College of Medicine, Seoul, South Korea

^c Department of Internal Medicine, The Armed Forces Capital Hospital, Seongnam, South Korea

^d Department of Internal Medicine and Cardiovascular Center, Seoul National University Hospital, Seoul, South Korea

* Corresponding author. Division of Allergy and Clinical Immunology, Department of Internal Medicine, Seoul National University College of Medicine, Seoul National University Hospital, 101 Daehak-ro, Chongno-gu, Seoul 110-744, South Korea. E-mail address: swj0126@gmail.com (W.-J. Song).

References

1. Spear GS. Eosinophilic explant carditis with eosinophilia: ?Hypersensitivity to dobutamine infusion. *J Heart Lung Transpl* 1995;14:755–60.
2. El-Sayed OM, Abdelfattah RR, Barcelona R, Leier CV. Dobutamine-induced eosinophilia. *Am J Cardiol* 2004;93:1078–9.
3. Takkenberg JJ, Czer LS, Fishbein MC, Luthringer DJ, Quartel AW, Mirocha J, et al. Eosinophilic myocarditis in patients awaiting heart transplantation. *Crit Care Med* 2004;32:714–21.
4. Aranda Jr JM, Woo G, Pauly DF, Rodriguez E, Hill JA, Schofield RS. Dobutamine-related asthma in a patient awaiting cardiac transplantation: the eosinophilic dilemma. *J Heart Lung Transpl* 2004;23:260–1.
5. Fenton M, Burch M, Sebire N. A dobutamine paradox: eosinophilic myocarditis in the explanted heart of a 9-year-old girl undergoing cardiac transplantation. *Cardiol Young* 2005;15:520–2.
6. Raju VP, Lewis NP, Katlapp G, Quader MA, Shah KB, Mankad AK. Dobutamine induced eosinophilic myocarditis and right heart failure requiring emergent biventricular assist device implantation. *ASAIO J* 2015;61:213–5.

^e Both authors equally contributed to this paper.

7. Lee CC, Luthringer DJ, Czer LS. Dobutamine-induced fever and isolated eosinophilic myocarditis in a 66-year-old male awaiting heart transplantation: a case report. *Transpl Proc* 2014;**46**:2464–6.
8. Picard D, Janela B, Descamps V, D'Incan M, Courville P, Jacquot S, et al. Drug reaction with eosinophilia and systemic symptoms (DRESS): a multiorgan antiviral T cell response. *Sci Transl Med* 2010;**2**: 46ra62.
9. Johnson MR. Eosinophilic myocarditis in the explanted hearts of cardiac transplant recipients: interesting pathologic finding or pathophysiologic entity of clinical significance? *Crit Care Med* 2004;**32**:888–90.
10. Vally H, Misso NL, Madan V. Clinical effects of sulphite additives. *Clin Exp Allergy* 2009;**39**:1643–51.
11. Funasako M, Funada A, Kanzaki H, Takahama H, Amaki M, Hasegawa T, et al. Eosinophilia associated with dobutamine allergy causes eosinophilic endomyocarditis confirmed by serial endomyocardial biopsies. *J Cardiol Cases* 2012;**6**:e179–82.
12. Halevy S, Cohen AD, Grossman N. Clinical implications of in vitro drug-induced interferon gamma release from peripheral blood lymphocytes in cutaneous adverse drug reactions. *J Am Acad Dermatol* 2005;**52**:254–61.
13. Martin M, Wurpts G, Ott H, Baron JM, Erdmann S, Merk HF, et al. In vitro detection and characterization of drug hypersensitivity using flow cytometry. *Allergy* 2010;**65**:32–9.

Received 17 January 2016
 Received in revised form 25 March 2016
 Accepted 31 March 2016
 Available online xxx