INTRODUCTION

Anaphylaxis is a severe and life-threatening hypersensitivity reaction, and its occurrence rate appears to be increasing.\(^1,2\) Antibiotics, especially β-lactams, nonsteroidal anti-inflammatory drugs (NSAIDs), and chemotherapeutic agents are often implicated in drug reactions;\(^2,3\) however, any medication or biological agent can potentially trigger anaphylaxis. For the assessment of drug-induced anaphylaxis, most physicians rely on a patient's clinical history and correlate the timing of symptoms with exposure. In vitro testing for drug allergy is necessary to determine the exact cause of the reaction, and to reduce the risk of re-exposure and prevent the unnecessary withdrawal of medications. Diagnostic tools have primarily focused on IgE-mediated anaphylaxis, and skin prick tests (SPTs), intradermal drug tests (IDTs), and drug-specific IgE testing have all been implemented.\(^4\) However, with the exception of several β-lactam antibiotics, pure reagents for in vivo or in vitro tests for most medications are not commercially available.

The basophil activation test (BAT) is a flow cytometry-based assay in which the expression of activation markers on the surface of basophils is measured following stimulation with an allergen. The BAT has been validated in IgE-mediated conditions, including food allergies, venom hypersensitivity, and pollen allergies.\(^5,6\)

In terms of drug allergies, the BAT may be useful for confirming clinical suspicions without performing dangerous drug challenge tests in cases where no alternative tests are available.

The aim of this study was to evaluate the clinical utility of this test in patients with drug-induced anaphylaxis. In total, 19 patients, all of whom had a history of moderate to severe anaphylaxis, were enrolled. None of the causative drugs had available in vitro tests or reliable skin tests; these drugs included, among others, first and second-generation cephalosporins, H2 blockers, and muscle relaxants. The BAT yielded positive results in 57.9% of the cases, which was similar to those results of skin prick and intradermal tests (42.1% and 57.9%, respectively). When basophils were double labelled with CD63 and CD203c, both of which are basophil activation markers, the positive rate was increased from 57.9% to 73.7%. Therefore, the results of this study confirm that the BAT is a quick, reliable, and safe diagnostic tool for patients with drug-induced anaphylaxis.

Key Words: Anaphylaxis; basophil; drug allergy
based on the criteria proposed at the second anaphylaxis sym-
pouum. In certain cases, oral chal-
lenge tests were performed with associated drugs, but not with
the suspected drug to ensure that they were tolerated, thus
 restricting causality to the suspected drug. Clinical characteristics
and demographic data were collected for each patient. Serum
total IgE was measured, and specific IgE was determined using
immunoCAP assays (Thermo Fisher Scientific Inc., Waltham,
MA, USA) when available. SPTs were performed for common
inhalant allergens (Allergopharma, Germany), and atopy was
defined as a positive SPT result. The study was approved by the
Hallym University Sacred Heart Hospital Ethics Committee,
and written informed consent was obtained from each subject.

**BATs**

Blood samples were drawn from the patients at least 2 weeks
post-hypersensitivity reaction. BATs were performed as pre-
viously described. Blood was collected in an EDTA tube, and red
cells were lysed using ammonium chloride lysis buffer
within 3 hours of sampling. After washing and re-suspending
the cells in PBS with 0.1% human serum albumin, the cells (200
µL) were stimulated (30 minutes, 37°C) with buffer containing a
positive control (anti-IgE 1 µg/mL; KPL, Gaithersburg, MD,
USA, A23187 3 µM; Sigma, St. Louis, MO, USA), a negative con-
trol (without any treatment), or serial dilutions of the drug (ap-
proximately, 8-10 dilution steps). Cells were then stained with 2
µL of a mixture containing anti-human CD123-FITC, anti-hu-
man HLA-DR-APC, anti-human CD63-PE-conjugated antibod-
ies (BD, Biosciences, San Jose, CA, USA), and anti-CD203c-PE-
conjugated antibodies (Beckman Coulter, Marseille Cedex,
France) for 40 minutes at room temperature. Basophil acti-
vation was evaluated by flow cytometric analysis (FACSCalibur;
BD, Immunocytometry Systems, San Jose, CA, USA). Results
are expressed as the percentage of CD63+ or CD203c+ baso-
phils; a stimulation index (SI) ≥2 and an absolute activated ba-
sophil percentage ≥5 were considered positive BAT responses.

**Statistical analysis**

All statistical analyses were performed using SPSS ver. 16.0
(SPSS Inc., Chicago, IL, USA). Because the number of subjects
was small, continuous variables were analyzed using the Mann-
Whitney U test, and categorical variables were analyzed using
Fisher’s exact test. A Spearman’s rank-order correlation test was
used to analyze changes in the basophil surface expression of
CD63 and CD203c. A P value <0.05 was considered significant.

**RESULTS**

**Baseline characteristics of study subjects**

The demographic and clinical characteristics of the study sub-
jects are shown in Tables 1 and 2. Nineteen patients, 9 men and
10 women, ranging in age from 21 to 70 years, were enrolled.
Twelve patients (63.2%) had a history of an allergic reaction to
the same culprit drug, and 5 experienced severe anaphylaxis.
Cephalosporin antibiotics were involved in 8 patients, raniti-
dine in 3, eperisone in 2, and streptomycin, insulin, propofol,
paclitaxel, tridol, and glimepiride in 1 patient each. The immu-
noCAP assays of penicilloyl G, penicilloyl V, amoxicilloyl, ampi-
cilloyl, and cefaclor were performed in patients with cephalo-
sporin antibiotics (Patient 1-8), however, only 1 patient (Patient
7) showed a positive result of cefaclor immunoCAP assay (9.96
KU/L, class 3).

**Table 1. Demographic features and clinical characteristics**

| Characteristic | n (%) |
|---------------|-------|
| Total number of patients | 19 |
| Gender, male | 9 (47.4) |
| Age, year, mean ± SD | 47.5 ± 11.8 (21-70) |
| Atopy, presence | 7/15 (46.7) |
| Past history of allergic diseases | 8 (42.1) |
| Symptoms at the time of prior exposure | 12 (63.2) |
| None | 7 (36.8) |
| Non-anaphylaxis allergic reactions | 3 (15.8) |
| Anaphylaxis | 9 (47.4) |
| Severity of anaphylaxis* | |
| Severe | 5 (26.3) |
| Moderate | 14 (73.7) |
| Latency period, minute, mean ± SD | 18.4 ± 12.1 (10-50) |

Values are given as number (%), unless otherwise indicated.
*The severity of anaphylaxis was graded as mild, moderate, or severe, using a
previously established grading system. Mild reactions were defined by presen-
tation of only cutaneous symptoms. Moderate symptoms were exhibited when
respiratory, cardiovascular, or gastrointestinal involvement was apparent;
whereas hypotension, hypoxia, loss of consciousness, or confusion was consid-
ered to reflect severe anaphylaxis (reference 7).

**Results of skin tests and BATs**

Eleven of 19 (57.9%) patients showed an upregulation of ba-
sophil cell surface CD203c and CD63 in response to drug stim-
ulation. The basophils of 14 patients (73.7%) showed an upreg-
ulation of 1 marker (CD63 or CD203c). There was a significant
positive correlation between the degrees of CD63 and CD203c
upregulation ($P=0.008$); however, the positive rates of the 2
markers were not significantly correlated ($P=0.181$). Five pa-
patients showed negative responses to both CD63 and CD203c,
and the other 6 patients showed discordant testing results (Ta-
ble 2). Fourteen patients (73.7%) had positive responses to ei-
ther the SPT or the IDT. Eight of 18 patients (42.1%) had posi-
tive responses to the SPT, and 11 of 14 patients (57.9%), exclud-
ing the 5 patients with severe anaphylaxis responses, had posi-
tive IDT responses.
Comparison of skin tests and BATs

The results of BATs and skin tests were not correlated in this study (P=0.530). All 3 patients with ranitidine-induced anaphylaxis (Patient 10-12 in Table 2) had severe reactions and positive SPTs results but no response to the BAT. One patient with cefaclor hypersensitivity (Patient 5) reacted positively only to the BAT and not to the SPT or to cefaclor-specific IgE. Each patient with eperisone- or propofol-induced anaphylaxis (Patient 13, Patient 16) had a negative response to the SPT, but the basophil expression of both CD203c and CD63 was upregulated.

DISCUSSION

For the diagnosis of drug-induced anaphylaxis, BATs yielded positive results in 57.9% of the cases, which was comparable to SPT and IDT rates (42.1% and 57.9%, respectively). The IDT was a sensitive diagnostic method in this study; however, discordances between the BAT results for the 2 markers were found in some patients, suggesting that the BAT may yield false positives or that basophil activation markers of CD63 and CD203c may have intra-individual variability. Although both CD63 and CD203c have been validated as acceptable markers in various allergic conditions. In a comparative study of the sensitivity of CD63 and CD203c in IgE-mediated amoxicillin allergies, CD203c sensitivity is superior to CD63. In contrast, patients with non-allergic NSAID hypersensitivity upregulated CD63 expression more frequently than CD203c expression. In this study, basophils of patients with cephalosporin-induced anaphylaxis upregulated CD203c, but not CD63. However, other drugs, including tridol and glimepiride, resulted in only CD63 upregulation, suggesting that different drugs regulate the expression of different markers.

Three patients (with reactions to cefaclor, eperisone, or propofol) had positive results only to the BAT; in these cases, both CD63 and CD203c were upregulated. The skin test results of these patients may have been false negatives, or another mechanism, such as non-IgE mediated or direct basophil activation,
may have been involved. In contrast, 3 cases had positive results only to the SPT, but not to the BAT, suggesting that these patients may react to ranitidine metabolites. Previous case reports have documented the positive results of skin tests to ranitidine and, as was the case with this study, the detection rate of serum specific IgE was relatively low compared to other skin tests.\textsuperscript{13-15} Taken together, these results suggest that ranitidine metabolites conjugated to body proteins may be involved in IgE-mediated reactions, even though the antigenic determinant of ranitidine remains unknown.

In conclusion, the BAT is particularly useful in patients with life-threatening anaphylaxis, in whom a drug provocation test is not advisable, and when other diagnostic tools are not possible. Although BAT has a false-negative risk due to the type of drug or drug metabolite, the diagnostic yield was increased in this study by the simultaneous measurement of CD63 and CD203c. In actual practice, the BAT can serve as a quick, reliable, and safe diagnostic tool.

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