Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.6296-20
Intern Med 60: 1573-1576, 2021
http://internmed.jp

【 CASE REPORT 】

Utility of Basophil Activation Test in a Case of Daisaikoto- and Yokukansan-induced Lung Injury

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Abstract:
Drug-induced lung injury is defined as a respiratory disorder. The usefulness of the basophil activation test (BAT) for drug allergy-related cases was recently reported. The patient was an 82-year-old woman who had been taking Daisaikoto and Yokukansan (herbal medicines) 3 months before developing dry cough. She was admitted to our hospital with an initial diagnosis of pneumonia with elevated serum LDH, KL-6, and IgE. Chest CT showed bilateral ground-glass opacities. Her bronchoalveolar lavage fluid showed increased eosinophils. Finally, a BAT was positive for both medications. Based on the findings, the patient was diagnosed with Daisaikoto- and Yokukansan-induced lung injury. The current case suggests that the BAT may be useful for the diagnosis of drug-induced lung injury.

Key words: drug-induced lung injury, BAT, DLST, herbal medicine, Kampo

(Intern Med 60: 1573-1576, 2021)
(DOI: 10.2169/internalmedicine.6296-20)

Introduction

Herbal medicine-induced lung injury was first reported in 1989 (1). Since then, there have been some reports of drug-induced lung injury due to herbal medicine; however, only a few of these were related to Daisaikoto and Yokukansan (2, 3). Daisaikoto and Yokukansan are frequently used for the improvement of neurosis and dementia-related mood disorders. Daisaikoto includes bupleurum, crow dipper, ginger, *Scutellaria baicalensis*, peony, jujube, bitter orange, and rheum. Yokukansan includes bupleurum, Chinese angelica, uncaria hook, cnidium rhizome, atractylodes lancea, poria cocos, and Chinese licorice. Thus, bupleurum is the only ingredient common to both herbal medicines. The diagnosis of drug-induced lung injury is based on domestic guidelines (4). There is no specific *in vitro* method to diagnose drug-induced lung injury; thus, the diagnosis is difficult to make. Recent findings have suggested the usefulness of a basophil activation test (BAT) in the diagnosis of drug-related allergy, due to the relatively low sensitivity and specificity of the conventional drug lymphocyte stimulation test (DLST) method (4-6). We herein report a case in which a diagnosis of drug-induced lung injury due to Daisaikoto and Yokukansan was made using a BAT and DLST.

Case Report

An 82-year-old woman was admitted to our hospital urgently due to an exacerbation of dyspnea with increased sputum production. She was a never-smoker, but she had been taking several medications, candesartan cilexetil, tiapride hydrochloride, quetiapine fumarate, galantamine hydrobromide, benidipine hydrochloride, ethyl icosapentate, camostat mesilate, mecobalamin, famotidine, limaprost alfadex for five years due to hypertension, type 2 diabetes, and chronic pancreatitis. Three months before her admission, she had been diagnosed by a primary care physician with Lewy body dementia and Daisaikoto and Yokukansan were prescribed for her dementia-related mood disorder.

On admission, her vital signs were almost stable (body temperature, 36.6℃; blood pressure, 150/84 mmHg; pulse, 68 beats/min), but her respiration rate was 20 breaths/min. Chest auscultation revealed bilateral fine crackles on the lower lungs. A physical examination revealed no evidence of congestive heart failure or other diseases, such as connective

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Received: September 14, 2020; Accepted: October 29, 2020; Advance Publication by J-STAGE: December 22, 2020
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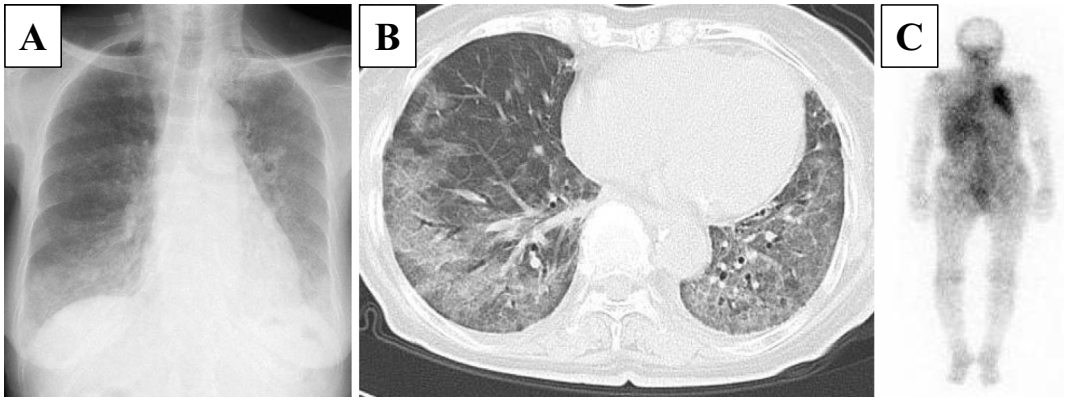

Figure 1. Clinical pictures before the initial treatment. (A) A chest X-ray showed diffuse ground-glass opacity (GGO) in both lung fields. (B) Chest CT on admission showed diffuse infiltrative shadows and GGO with air-bronchogram on the dorsal side of both lower lobes. (C) Gadolinium scintigraphy showed accumulation in the left upper and middle lung field, and lower right lung field.

tissue disease. Chest X-ray and high-resolution computed tomography (HRCT) showed diffuse infiltrative shadows and ground-glass opacities with air-bronchograms on the dorsal side of both lower lobes (Fig. 1A, B). Gadolinium (Ga) scintigraphy showed accumulation in the left upper and middle lung field, and lower right lung field (Fig. 1C). Laboratory tests showed elevated serum levels of lactate dehydrogenase (LDH; 429 mg/dL), C-reactive protein (CRP; 7.1 mg/dL), Krebs von den Lungen-6 (KL-6; 1,811 mg/dL), pulmonary surfactant protein-D (SP-D; 317.7 mg/dL), and IgE (169 IU/dL; measured specific IgE was negative) with hypoxemia. A pulmonary function test showed restrictive impairment.

On the 3rd day of hospitalization, a bronchial alveolar lavage (BAL) test was performed from the right B4 (recovery rate: 30 mL/150 mL). The bloody BAL fluid showed 3.0% lymphocytes (CD 4/8=0.356) and increased eosinophils (39%). A BAL fluid culture did not show any bacterial organisms. Transbronchial lung biopsy was not performed due to acute hypoxemia during the BAL procedure. The DLST and BAT were performed for Daisaikoto and Yokukansan using peripheral blood before any additional treatment. In the tests for Yokukansan, The DLST was weakly positive (S.I. 2.5), while the BAT was positive (Fig. 2A). Both tests were positive for Daisaikoto (DLST: S.I. 16.3; Fig. 2B). After excluding other types of pneumonitis, we concluded that the diagnosis was Daisaikoto- and Yokukansan-induced lung injury and that the causative ingredient was possibly bupleurum.

Daisaikoto and Yokukansan were discontinued immediately upon admission and systematic corticosteroid therapy with prednisolone (0.5 mg/kg/day), was started after a bronchoscopic examination and blood tests. The patient's symptoms and laboratory findings showed improvement immediately after the cessation of herbal medicine and the start of treatment. The prednisolone dose was tapered with no recurrence of lung injury. She was discharged from our hospital on the 30th day of hospitalization. We instructed her to refrain from using any herbal medicine after her discharge.

Discussion

In the present case, Daisaikoto-and Yokukansan-induced lung injury was observed with eosinophilic inflammation. The BAT was positive for both drugs, while the DLST was only positive for Daisaikoto. Since bupleurum was the only ingredient common to both drugs, it was considered to be the most likely cause of the patient's condition in the current case. Recent findings have suggested the usefulness of the BAT in the diagnosis of drug-related adverse events, as was shown in the current case (5).

Recently, the number of reports on herbal medicine-induced lung injury in Japan have been increasing (3). According to a review of herbal medicine-induced lung injury, Shosaikoto is the most common causative drug (26%), followed by Saireito (16%), Seishinrenshiin (8%), and Bofutsusyosan (8%) (2). Yokukansan has not previously been reported as a cause of lung injury; however, Daisaikoto was reported as a cause of lung injury in 1 of 73 patients (2). Importantly, bupleurum is included in 59% of herbal medicines that are reported to have caused lung injury (2). Other ingredients, such as skullcap (86%), licorice (85%), and ginseng (62%) are the top 3 ingredients related to drug-induced lung injury, but these ingredients are not included in Daisaikoton or Yokukansan (2).

Five conditions stated by Japanese Respiratory Society must be met to diagnose drug-induced lung injury: 1) history of drug intake, 2) clinical manifestations, 3) exclusion of other causes, 4) symptom improvement after drug discontinuation, and 5) symptom recurrence after re-administration (a so-called challenge test) (4). The current case met four of the criteria; we did not re-administer the causative drug. The challenge test is still considered to be the gold standard for the diagnosis of drug-induced lung injury; however, it is not performed in many cases due to the risk, especially in elderly patients with hypoxia. Since the mechanisms of herbal

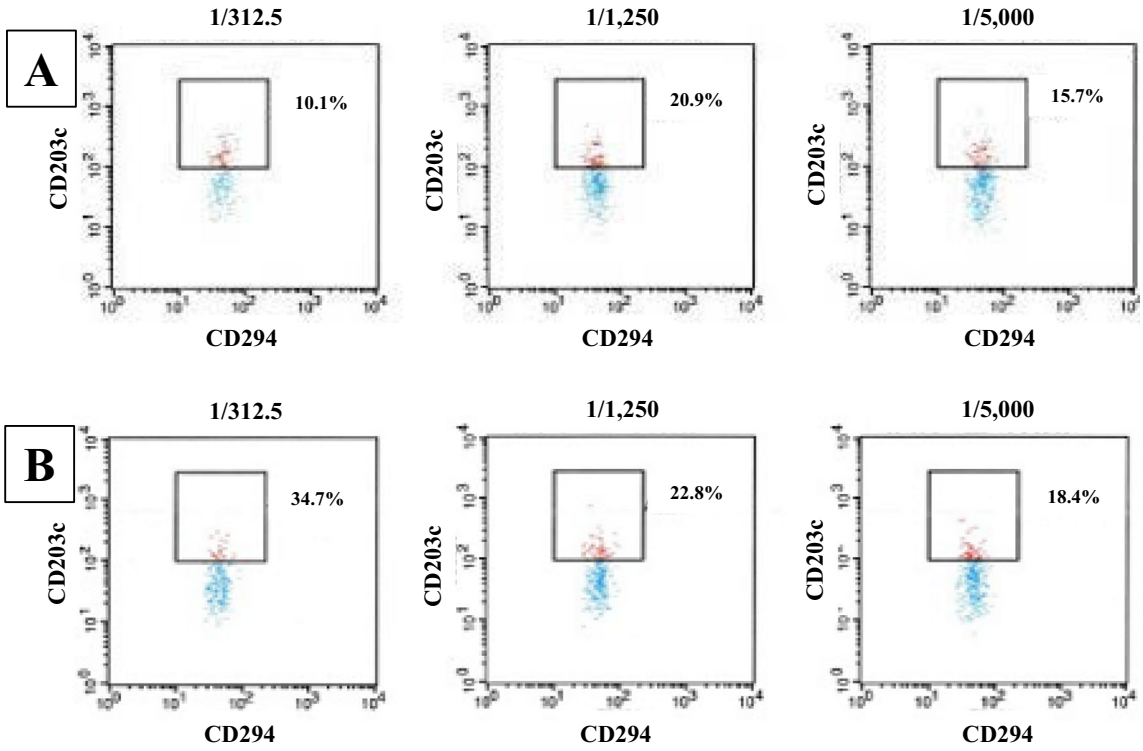

Figure 2. **Results of the basophil activation tests for (A) Yokukansan and (B) Daisaikoto. CD3-, CD294 (CRTH2)+, FcεRI+, and CD203c+were used to determine the activation of basophils. Concentrations of 1:312.5, 1:1,250, and 1:5,000 were used for both herbal medicines. The percentages of activated basophils are shown next to the box of each figure and the values of the positive and negative controls were 27.4% and 1.0%, respectively.**

medicine-induced lung injury are thought to be due to an allergic reaction (type I, III, and IV based on the classification of Cooms and Gell), rather than a toxic reaction, therefore an *ex-vivo* model was proposed to diagnose (4, 7). DLSTs are often used and mostly reflect type IV reactions; however, the specificity and sensitivity are relatively low due to the delicate testing processes (4, 7, 8). A previous report showed false-positive DLST results for *Angelicae radix* and *Scutellariae radix* even in subjects who had never taken any herbal medicines (8). DLSTs were reported to show positive results in 67.6% of cases of herbal medicine-induced lung injury (9). Recently, the BAT was introduced in this field for the diagnosis of medication-induced allergic reaction (5). The BAT examines the activation of IgE-positive basophils, which are theoretically involved in type I allergic reactions. Activated basophils are gated using the following features CD3-, CD294 (CRTH2)+, FcεRI+, and CD203c+. IgE was used as a positive control in our current test. Recent reports demonstrated that for the diagnosis of allergic reaction to beta-lactam antibiotics, the BAT showed higher the sensitivity and specificity in comparison to the measurement of the specific IgE value (5, 6, 10). Other than antibiotics, the BAT was showed 36-92% sensitivity and 81-100% specificity for neuromuscular blocking agents (6). These results suggested that BAT could be an option for the diagnosis of drug-related allergy since there is still no gold-standard *ex vivo* test. Several reports suggested the usefulness of BAT for the diagnosis of IgE-independent delayed food allergy and drug eruption, but underlying mechanisms are still no clear. There are several limitations regarding the use of the BAT in the diagnosis of drug-induced lung injury: 1) the protocol for BAT may differ between facilities (e.g., positive and negative cut-off values), 2) there is less evidence to support the use of the BAT for the diagnosis of drug-induced lung injury, 3) a negative test result does not rule out the possibility of a reaction to drug metabolites. Also, the use of CD203c, rather than CD63, for the BAT may give different results (5); however, others have shown that similarity between the two tests (11, 12). Regarding with the cut-off value, we used the current protocol to test small numbers of healthy subjects and the results were negative. Furthermore, the incubation of both herbal medicines with basophils for 1 hour yielded negative results in our current case. Specifically, basophil activation was seen in 0-2.4% of the total cells at the 1-hour time point. Therefore, we briefly set 1% as the cut-off value. Since eosinophils were dominant in BAL fluid, it suggested that related chemokines may have played an important role in our current case (13). To date, the BAT is the only method for the diagnosis of a basophil-related drug reaction, which may occur in patients with drug-induced lung injury, and further studies are needed to investigate the relevance of this use of the test.

Bupleurum, 190 species of which exist around the world, is one of the most commonly used plant-extracted ingredi-

ents in prescription herbal medicines. The main chemical ingredient within bupleurum is saikosaponin, which is present as saikosaponins a, c, d, and e (14, 15). Other reports have shown a broad range of pharmacological effects of saikosaponins, including anti-pyretic, anti-viral, anti-inflammatory, and anti-tumor effects (14, 15). Although previous studies observed reported the inhibition of lymphocytes, in the current case, the DLST detected lymphocyte proliferation. The amount of bupleurum in Daisaikoto is twice that in Yokukansan, which may have altered the results of DLST. Based on the BAT for Daisaikoto, which was performed in the same manner, it was suspected that type I and IV allergic reactions were both involved (5, 7). Since the sensitivity of the DLST or BAT alone is not sufficient for the diagnosis of herbal medicine-induced lung injury, the combination of both tests seemed to be reasonable as it would include more mechanistic aspects of the disease manifestation.

In the diagnosis of drug-induced lung injury, *ex vivo* testing with a BAT may yield useful information, especially for elderly patients who cannot tolerate re-admission of the suspected drug. Further investigation is warranted to determine the usefulness of the BAT and the role of basophils in this field.

The authors state that they have no Conflict of Interest (COI).

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