Short Report

The association of lymphocyte phenotypes and outcomes after discontinuing eltrombopag in ITP

Satoko Oka[1], Kazuo Ono[2], and Masaharu Nohgawa[1]

1 Division of Hematology, Japanese Red Cross Society Wakayama Medical Center, Wakayama, Wakayama, Japan

2 Division of Pathology, Japanese Red Cross Society Wakayama Medical Center, Wakayama, Wakayama, Japan

Correspondence to: Satoko Oka M.D., Division of Hematology, Japanese Red Cross Society Wakayama Medical Center, Wakayama, Wakayama, Japan

Phone: +81-734-22-4171, Fax: +81-734-21-8132, E-mail: okas@jasmine.ocn.ne.jp

Abstract

Eltrombopag is a highly effective treatment for immune thrombocytopenia (ITP). Cases of durable remission after the discontinuation of eltrombopag in adult ITP have recently been reported; however, the frequency and mechanisms responsible for this phenomenon remain unknown. In the present study, we examined the phenotypes of lymphocytes in ITP to clarify whether they predict outcomes after the discontinuation of eltrombopag. We examined 56 adult newly diagnosed ITP patients treated with eltrombopag after a median time from diagnosis of 48 months. Among the 38 patients who achieved complete remission, eltrombopag was discontinued in 26. Among the 26 patients, 12 (46.2%) had an immediate relapse after discontinuing eltrombopag and 16 (53.8%) showed sustained response without additional ITP therapy, despite discontinuing eltrombopag, with a median follow-up of 52 months. No significant differences were observed in platelets, the median duration of eltrombopag, the absolute number of T, B, and NK cells at the initiation of eltrombopag between patients who sustained response and those who relapsed after discontinuing eltrombopag. However, the number of B and NK cells at the discontinuation of eltrombopag was higher in patients who sustained response than in those who relapsed ($p=0.022$ and $p=0.012$, respectively).

The present results indicate that the absolute number of B ($\geq 0.20 \times 10^9$/L) and NK ($\geq 0.36 \times 10^9$/L) cells at the discontinuation of eltrombopag contributes to the prediction of outcomes.

Key words: immune thrombocytopenic purpura (ITP), eltrombopag, discontinuation, B cell, NK cell

Introduction

Immune thrombocytopenic purpura (ITP) is an immune-mediated bleeding disorder characterized by the presence of plasma antibodies recognizing platelet autoantigens and their destruction by macrophages in the spleen (1). The thrombopoietin receptor agonists (TPO-RAs), eltrombopag and romidepsin, have been consolidated as second or third-line anti-ITP therapy (2, 3). In pivotal clinical trials, reported response rates to eltrombopag ranged between 59 and 88% and the treatment was tolerated well, but was an expensive treatment option (4). Short- and medium-term treatments with eltrombopag not only reduce costs, but may also decrease the risk of side effects. However, the frequency of a sustained response after discontinuing eltrombopag without additional therapy for ITP and predictive factors of a sustained response

currently remain unknown. We conducted the present study to elucidate the relationship between the phenotypes of lymphocytes in ITP and whether they predict outcomes after the discontinuation of eltrombopag.

Patients and methods

We retrospectively analyzed 56 adult ITP patients treated with eltrombopag at our hospital between January 2008 and January 2020. Patients, disease characteristics, and clinical data were recorded. Eltrombopag was administered at doses approved by the guidelines (5). Primary ITP was defined as a platelet count $<100 \times 10^9$/L in the absence of other causes or disorders that may be associated with thrombocytopenia. The phases of ITP were categorized as newly diagnosed ITP (≤3 months), persistent ITP (3 months to 1 year), and chronic ITP (> 1 year), according to the criteria of the ITP international working group consensus. Treatment responses were evaluated according to the consensus definition of the International Working Group (6). A "complete response (CR)" was defined as a platelet count equal to or greater than 100×10^9/L and the absence of bleeding. A "response (R)" was defined as a platelet count equal to or greater than 30×10^9/L and at least a 2-fold increase in the baseline count and absence of bleeding. "No response (NR)" was defined as a platelet count lower

than 30×10^9/L or less than a 2-fold increase in the baseline platelet count or bleeding. In the present study, we defined the successful discontinuation of eltrombopag for those patients (sustain response) who reached CR and maintained this response for at least 6 months after discontinuation without additional anti-ITP therapy. The discontinuation of eltrombopag was at the treating physician's discretion.

Peripheral blood specimens were collected in EDTA tubes. After incubation of whole peripheral blood with monoclonal antibodies for 15 minutes at room temperature in dark, erythrocytes were lysed with NH4CL for 10 min, followed by two washing steps using phosphate buffered saline solution. The cells were the resuspended and fixed with 1% paraformalddehyde. Four-color flow cytometry was performed using Navios (Beckman-Coulter). Four-color direct immunofluorescent staining was performed as described by the manufacturer, using a 7-tube panel. 100μL blood samples, containing EDTA, were transferred to tubes and the tubes were placed in the lyses Wash Assistant. Fluorescent labeled monoclonal antibodies were used to detect cell surface antigens: anti-CD45 ECD, anti-CD19-FITC, anti-CD3 PC5, anti-CD56 PE and anti-CD16 PC7. Lymphocyte subpopulations were characterized by combining monoclonal antibodies for T(CD45+, CD3+), B (CD45+, CD19+)and NK cells(CD45+, CD56+, CD3-, CD16+). Cells were collected and analyzed on a Navios (Beckman-

Coulter) using Kaluza software (Beckman-Coulter). The tubes were aspirated until dry to maximize cell yield. Lymphocytes were identified in the standard manner based on CD45 expression and side angle light scatter. Peripheral blood was obtained from 10 healthy donors (5 males and 5 females; age range, 34 – 65 years). Platelet counts ranged between 185 and 250 × 10^9/L (median, 221 × 10^9/L). The present study was conducted in accordance with the principles of the Declaration of Helsinki. This study was approved by the institutional ethics committee.

Statistical analysis

Fisher's exact and Mann-Whitney U tests were used to assess the significance of differences, with a *p* value less than 0.05 being regarded as significant. Statistical analyses were performed with the SPSS 11.5 system.

Results

Patient characteristics were retrospectively evaluated, including age, gender, months with ITP, type of therapies received prior to the initiation of eltrombopag, and lymphocyte and platelet counts. As shown in Table 1, 56 patients who received eltrombopag were examined, and included 20 males (35.80%) and 36 females (64.20%) with a median age of 68 years (range 31-92 years). The median time from diagnosis to the initiation of eltrombopag was 44 (range, 1- 132) months. The median

number of previous therapies was 2 (0-4), and no patients had undergone splenectomy. The lymphocyte count in peripheral blood at the initiation of eltrombopag was $1.2\pm0.5\times10^9$/L. The absolute number of T, B and NK cells were $1.1\pm0.1\times10^9$/L, $0.28\pm0.02\times10^9$/L and $0.38\pm0.01\times10^9$/L, respectively. The initial response rate to eltrombopag was 92.8%, including 67.8% CR. The median duration of the eltrombopag treatment was 41 (range, 3-91) months.

Eltrombopag was discontinued in 28 out of 38 (74%) patients who achieved CR. The reasons for this were a persistent response over time despite a decrease in the dose (n= 21), a platelet count > 400×10^9/L (n=4), patient's request (n=2), and unknown (n= 1). Among the 28 patients, 12 (46.2%) had an immediate relapse after discontinuing eltrombopag, which required rescue therapy. Sixteen patients (53.8%) showed sustained response without additional ITP therapy, despite the discontinuation of eltrombopag, with a median follow-up of 42 months (range, 14-120) months. As shown in Table 2, no significant differences were observed in age, gender, the duration of ITP, prior number of therapies, previous treatments, or platelet counts at the initiation of eltrombopag between patients who sustained response and those who relapsed after discontinuation of eltrombopag. Furthermore, no significant differences were observed in the absolute number of T, B, and NK cells at the initiation of

eltrombopag between these two groups. No significant differences were noted in platelet counts at the discontinuation of eltrombopag between two groups. However, the absolute number of B and NK cells at the discontinuation of eltrombopag was higher in patients who sustained response than in those who relapsed after stopping eltrombopag ($p=0.022$ and $p=0.012$, respectively). The absolute number of CD19+ and NK cells at the discontinuation of eltrombopag in patients who sustained CR/R was similar to that in normal controls (Figure 1).

Discussion

Although the use of TPO-RAs as a second- or third-line therapy for ITP has increased, there are still no guidelines regarding the duration of TPO-RAs in patients achieving CR. Recent studies showed that a large proportion of patients with chronic ITP treated with TPO-RAs achieved a lasting response after treatment discontinuation (7, 8). A French observational study reported that 53% (8/14) of patients sustained response despite the discontinuation of TPO-RAs, with a median follow-up of 13.5 months (7). According to Gonazalez-Lopez, 53% (26/49) of patients achieved sustained response (8). Approximately 50% of patients sustained response despite the discontinuation of TPO-RAs; however, there are currently no reliable factors for

predicting a sustained response after the discontinuation of eltrombopag. In the present study, the sustained response rate after the discontinuation of eltrombopag (53.8%) was consistent with previous findings. In patients who sustained response, the absolute number of B and NK cells was higher at the discontinuation of eltrombopag than at its initiation, and was similar to those in the control group. However, no significant differences were observed in the number of B and NK cells between the initiation and discontinuation of eltrombopag in the relapse group. NK cell numbers and functions are reduced in autoimmune diseases and may play an immunoregulatory role in disease pathology (9). Semple et al. reported increased anti-platelet Th activity and platelet destruction in association with NK cell activity defects (10). NK cells exert suppressive effects on antibody production and play an important role in B cell regulation . The present results revealed a relationship between NK cells and the pathogenesis of ITP.

In conclusion, the present results showed that the absolute number of B ($\geq 0.20 \times 10^9$/L) and NK ($\geq 0.36 \times 10^9$/L) cells at the discontinuation of eltrombopag may contribute to predicting outcomes after the discontinuation of eltrombopag. The main limitation of the present study is its retrospective design. In order to elucidate this

phenomenon in more detail, further studies are needed to confirm lymphocytes in ITP patients treated with eltrombopag.

Acknowledgments

None

Conflicts of interest statement

All authors declare no conflicts of interest.

Author Contributions

Satoko Oka involved in the conception and design of this study. Kazuo Ono and Masaharu Nohgawa were involved in the acquisition of data.

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Table legends

Table 1. Characteristics of patients treated with eltrombopag.

Table 2. Characteristics of patients who sustained response and those who relapsed after discontinuing eltrombopag.

Figure 1. The number of CD3, CD19, and NK cells at the initiation and discontinuation of eltrombopag in patients who sustained response (a) and those who relapsed (b) after discontinuing eltrombopag.