Azathioprine Intolerance in Japanese Patients with Antineutrophil Cytoplasmic Antibody-associated Vasculitis

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Abstract

Objective To assess the safety of azathioprine (AZA) in Japanese patients with antineutrophil cytoplasmic antibody-associated vasculitis (AAV).

Methods We retrospectively enrolled 67 consecutive AAV patients who had initiated AZA treatment from January 2006 to August 2014 at Okayama University Hospital. We evaluated the development of severe adverse events (AEs), AZA discontinuation due to total AEs (severe AEs included) within 1 year, and AZA-associated risk factors.

Results The patients’ median age was 70 years old. Forty-nine women and 18 men participated at the initiation of the study. Fifty-eight (87%) patients experienced AEs, and 36 experienced severe AEs (21 hepatic and 11 cytopenic severe AEs). Thirty-one (46%) patients discontinued treatment because of AEs. Abnormal hepatic laboratory test results at the treatment initiation were more frequent in patients with hepatic severe AEs and were associated with treatment discontinuation. The leukocyte and neutrophil counts at the treatment initiation were lower in the patients who discontinued treatment because of cytopenic AEs than in those who continued treatment. Only two patients experienced flare-ups during treatment.

Conclusion The AE-associated AZA discontinuation rate in Japanese AAV patients was relatively high. AZA use warrants caution in patients with abnormal hepatic laboratory test results or low leukocyte or neutrophil counts.

Key words: adverse events, anti-neutrophil cytoplasmic antibody-associated vasculitis, azathioprine

Introduction

Antineutrophil cytoplasmic antibody (ANCA)-associated vasculitis (AAV) is a systemic disorder associated with ANCA that predominantly affects small vessels and is classified into microscopic polyangiitis (MPA), granulomatosis with polyangiitis (GPA), and eosinophilic granulomatosis with polyangiitis (EGPA) (1). A nationwide prospective inception cohort study in Japan revealed that AAV classification, the type of organs involved, the age of onset, and the treatment patterns were different from those in Western countries (2, 3). Azathioprine (AZA) is a widely used drug for the treatment of autoimmune diseases. AZA has been used as a standard AAV treatment following successful remission with cyclophosphamide (CYC) (4-6). However, toxic adverse events (AEs), including nausea, vomiting, myelosuppression, and hepatotoxicity, develop frequently and limit the clinical benefits of AZA. According to clinical trials of AAV, the incidence of myelotoxicity was 16-34% and 1.7-11% in patients who discontinued AZA because of AEs, such as hepato-
Data collection

The data at the time of the diagnosis of each patient included their demographic information, disease activity using Birmingham Vasculitis Activity Score (BVAS) (17), and ANCA positivity. At the initiation of the AZA treatment, the following data were collected: purpose of the administration, dosage of AZA, the concomitant use of drugs, laboratory data, previous treatments, and concomitant dosage of glucocorticoids. The dosage of AZA was adjusted at the discretion of the attending physicians. Abnormal hepatic laboratory test results at the initiation of the treatment were defined as an aspartate aminotransferase (AST), alanine aminotransferase (ALT), or \( \gamma \)-glutamyltranspeptidase (\( \gamma \)-GTP) level exceeding the normal range of our hospital. An abnormal renal laboratory test result at the initiation of the treatment was defined as an eGFR less than 60 mL/min/1.73 m\(^2\).

Outcomes

The primary safety outcomes of this study were the development of severe AEs and the discontinuation of AZA due to AEs within one year of treatment. Severe AEs were important as a highly reproducible outcome based on distinct definitions, while the discontinuation of AZA was important as a clinically relevant outcome. We extracted information on the AEs from the medical charts and laboratory data. Hepatic AEs were evaluated by determining the AST, ALT, and \( \gamma \)-GTP levels, and cytopenic AEs were evaluated by determining the WBC and platelet (PLT) counts and hemoglobin (Hb) level. Renal AEs were evaluated by determining the serum creatinine level, while other AEs were evaluated according to the medical chart records. AEs were categorized according to the Common Terminology Criteria for Adverse Events (CTCAE, version 4.0; http://evs.nci.nih.gov/ftp1/CTC AE/About.html) (Supplemental Data). Severe AEs were originally defined as new or worsened AEs compared to those at baseline that were classified higher than grade-2 severity.

The secondary outcome was relapse, which was defined as the recurrence or worsening of disease activity (BVAS >0 and/or interstitial pneumonia) and/or elevated C-reactive protein (CRP) level without other causes.

Statistical analyses

Continuous variables were compared using Student’s t-test or the Mann-Whitney U test as appropriate, and categorical variables were compared using a chi-squared test. The tests were two-tailed, and differences at p<0.05 were considered significant. All statistical analyses were performed using the Statistical Package of JMP for Windows software program, version 11.0.2 (SAS Institute, Cary, NC, USA).
Hb level, and PLT count were 7,890/μL (IQR, 6,910-9,400), (IQR, 11-24), 23 U/L (IQR, 14-41), and 48.2 mL/min/1.73 γ spectively, at the initiation of AZA. The median AST, ALT, results were found in 18 (27%) and 45 (67%) patients, re-
tantly, and sulfamethoxazole/trimethoprim was used in 43 (IQR, 7.5-12.5). Antihyperuricemics was not used concomi-
tients). The concomitant prednisolone dose was 10 mg/day
patients, 50 mg/day in 30 patients, 75 mg/day in 4 patients, and 100 mg/day in 11 patients).

**Table 1. All Adverse Events.**

<table>
<thead>
<tr>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
<th>Grade 5</th>
<th>Unclassifiable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal hepatic laboratory test results</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Cytopenia</td>
<td>20</td>
<td>10</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal renal laboratory test results</td>
<td>17</td>
<td>9</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infection</td>
<td>2</td>
<td>3</td>
<td>3</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Gastrointestinal symptoms</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hair loss</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin cancer</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unspecific symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
</tbody>
</table>

The severities were scored using the Common Terminology Criteria for Adverse Events version 4.0 (http://evs.nci.nih.gov/ftp1/CTCAE/About.html) (Supplemental data).

* aDizziness occurred in one patient, and dry mouth, insomnia, and light-headedness in the other patient.

**Results**

**Patient characteristics and treatment effectiveness**

Of the 67 AAV patients included in the study, 34 were classified as MPA, 13 as GPA, 3 as EGPA, and 17 as un-
classifiable AAV. At the diagnosis of AAV, the median age of all patients was 69 years [interquartile range (IQR), 63-
76], and 49 (73%) patients were women. Myeloperoxidase (MPO)-ANCA and proteinase 3 (PR3)-ANCA were positive
in 53 (82%) and 5 (8%) patients, respectively. The BVAS was 14 (IQR, 10-19), and the organs involved were ear, nose,
and throat (13%), chest (21%), kidneys (76%), and nervous system (42%). A median dose of 40 mg of predni-
solone was initiated as remission induction therapy, and con-
comitant CYC was used in 51 of the 67 (76%) patients. AZA was initiated for remission maintenance in 48 of 67
(72%) patients and for remission induction in 19 (28%). Of the 48 patients in whom AZA was initiated for remission
maintenance, 42 had been treated with CYC, and 6 had never been treated with a concomitant immunosuppressant.

Of the 19 patients subjected to remission induction, 4 received this therapy because of difficulty in CYC continu-
ation, and 10 received it at relapse. In 5 newly diagnosed patients subjected to remission induction, general, cutaneous,
nervous system, renal, and chest (including interstitial pneu-
monia) manifestations were observed in 3, 2, 2, 1, and 1 patients,
respectively. At treatment initiation, the median age was 70 years (IQR, 63-76), and the median initial AZA dose
was 0.63 mg/kg/day (IQR, 0.53-0.99; 25 mg/day in 36 pa-
tients, 50 mg/day in 28 patients, and 100 mg/day in 3 pa-
tients). The concomitant prednisolone dose was 10 mg/day
(IQR, 7.5-12.5). Antihyperuricemics was not used concomi-
tantly, and sulfamethoxazole/trimethoprim was used in 43
(64%) patients. Abnormal hepatic and renal laboratory test
results were found in 18 (27%) and 45 (67%) patients, re-
spectively, at the initiation of AZA. The median AST, ALT,
γ-GTP, and eGFR values were 18 U/L (IQR, 14-25), 17 U/L
(IQR, 11-24), 23 U/L (IQR, 14-41), and 48.2 mL/min/1.73
m² (IQR, 36.8-63.9), respectively. The median WBC count,
Hb level, and PLT count were 7,890/μL (IQR, 6,910-9,400),
11.5 g/dL (IQR, 10.9-12.5), and 25.4×10⁹/μL (IQR, 21.9-
33.4×10⁹), respectively. The median CRP level was 0.12 mg/
dl. (IQR, 0.03-0.42). The maximum dose of AZA for 1 year
was 0.98 mg/kg/day (IQR, 0.58-1.20; 25 mg/day in 22 pa-
tients, 50 mg/day in 30 patients, 75 mg/day in 4 patients,
and 100 mg/day in 11 patients).

**Adverse events with AZA treatment**

During the observational period, 58 (87%) patients (re-
mission maintenance initiated in 42 patients and remission
induction in 16) experienced AEs (105 events). The details
and severity scores according to CTCAE v4.0 are shown in
Table 1. Of those 58 patients, 36 (62%) experienced severe
AEs according to our definitions. The characteristics of the
patients who experienced frequent severe AEs were com-
pared to those of a control group of 25 patients who did not
experience severe AEs (non-severe AE group). Six patients
were excluded from this analysis because their severities
were unclassifiable.

In the 21 patients with severe hepatic AEs (hepatic severe
AE group), abnormal hepatic laboratory test results at the
initiation of the treatment were more frequent than in the
non-severe AE group (p=0.047, Table 2). The median AST,
ALT, and γ-GTP levels were also significantly higher in the
hepatic severe AE group than those in the non-severe AE
group (p=0.0018, p=0.02, and p=0.0089, respectively; Ta-
ble 2).

No significant differences were found between the 11 pa-
tients with cytopenic severe AEs and the non-severe AE
group (Table 2).

**Discontinuation of AZA**

AZA was discontinued in 31 patients (46%) because of
AEs within a median of 48 days (IQR 22-91) from starting
treatment, and because of abnormal hepatic laboratory test
results (12 patients), cytopenia (12 patients), infection (3 pa-
tients), gastrointestinal symptoms (3 patients), and other ab-
normalities (alopecia: 1 patient, skin cancer: 1 patient, and
unspecific symptoms: 2 patients). AZA was discontinued in
22 (61%) of the 36 patients with severe AEs. To compare
the characteristics between patients who discontinued and
continued treatment, we set the group of 35 patients who
patients after AZA discontinuation (p=0.010).

<table>
<thead>
<tr>
<th>Age, median (IQR), year</th>
<th>Hepatic severe AE (n=21)*</th>
<th>Cytopenic severe AE (n=11)*</th>
<th>Non-severe AE (n=25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female, n (%)</td>
<td>70 (65-75)</td>
<td>68 (63-71)</td>
<td>70 (64-80)</td>
</tr>
<tr>
<td>Maximum daily dose of AZA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25 mg/day, n</td>
<td>5</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>50 mg/day, n</td>
<td>10</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>75 mg/day, n</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>100 mg/day, n</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Abnormal hepatic laboratory test results, n (%)</td>
<td>10 (48)*</td>
<td>4 (36)</td>
<td>5 (20)</td>
</tr>
<tr>
<td>Abnormal renal laboratory test results, n (%)</td>
<td>14 (67)</td>
<td>9 (82)</td>
<td>15 (60)</td>
</tr>
<tr>
<td>Hb, median (IQR), g/dL</td>
<td>11.8 (10.9-12.6)</td>
<td>10.9 (10.7-11.4)</td>
<td>11.5 (10.9-13.2)</td>
</tr>
<tr>
<td>PLT, median (IQR), ×10^9/L</td>
<td>25.1 (22.2-34.3)</td>
<td>25.1 (19.1-28.7)</td>
<td>24.7 (20.7-31.8)</td>
</tr>
<tr>
<td>AST, median (IQR), U/L</td>
<td>25 (18-31)*</td>
<td>20 (13-30)</td>
<td>15 (14-20)</td>
</tr>
<tr>
<td>ALT, median (IQR), U/L</td>
<td>24 (15-31)*</td>
<td>21 (8-26)</td>
<td>17 (11-21)</td>
</tr>
<tr>
<td>γ-GTP, median (IQR), U/L</td>
<td>35 (24-75)*</td>
<td>31 (16-58)</td>
<td>19 (14-33)</td>
</tr>
<tr>
<td>eGFR, median (IQR), mL/min/1.73 m²</td>
<td>48 (40.5-70.9)</td>
<td>40.7 (32.4-59.8)</td>
<td>53.3 (38.7-62.3)</td>
</tr>
</tbody>
</table>

Severe AEs were originally defined as new or worsened AEs compared to those at baseline, and which were classified higher than grade-2 severity.

*p<0.05 in the comparison between the severe hepatic AE and non-severe AE group.


Table 2. Demographic and Laboratory Data at the Initiation of the AZA Treatment in the Hepatic severe AE, Cytopenic severe AE, and Non-severe AE Group.

To our knowledge, this study is the first to focus on the safety of AZA in AAV patients. AZA was initiated for maintenance therapy in 72% of the patients enrolled in the study, and concomitant CYC was used for remission induction in 76% of the enrolled patients. AEs developed in 58 of the 67 patients, 36 of whom experienced severe AEs. AZA treatment was discontinued in 46% of the patients because of AE development within 1 year of treatment. Treatment discontinuation due to hepatic AEs and cytopenic AEs was more frequent in the patients with abnormal hepatic laboratory test results at the initiation of the treatment. Approximately half of the patients who discontinued treatment because of cytopenic AEs experienced cytopenic severe AEs. The other patients discontinued the treatment because of mild cytopenic AEs. Leukocyte and neutrophil counts at the initiation of treatment were significantly lower in the patients who discontinued treatment because of cytopenic AEs (p=0.016 and p=0.013, respectively) than in those who continued treatment (Table 3). Of the 12 patients who discontinued AZA because of cytopenic AEs, 9 were treated with CYC prior to the initiation of AZA. The median time from the last CYC administration to AZA discontinuation because of cytopenic AEs was 105 days (IQR 64.5-351.5).

After AZA discontinuation, 27 patients were treated with glucocorticoids alone, 3 with mizoribine, and 1 with mycophenolate mofetil. Two (6%) of 36 patients experienced flare-ups during AZA treatment compared to 9 (29%) of 31 patients after AZA discontinuation (p=0.010).
these low AZA treatment discontinuation rates in previous AAV and SLE studies, we conclude that the high discontinuation rate of AZA in the present study was not caused by disease specificity but by other factors.

The majority of patients enrolled in the present study experienced AZA-related AEs; hepatic AEs were observed in 45% of the patients, while this rate ranged from 3.8-6.3%, 3.8-21.4%, and 16% in previous studies of AAV (5, 6), SLE (18, 22), and IBD (23), respectively. The abnormal hepatic laboratory test results found at the initiation of the present AZA treatment may have been an effective predictor of hepatic severe AEs and AE-related discontinuation of AZA treatment. In previous clinical trials, patients presenting with abnormal hepatic laboratory test results were excluded from the study; therefore, the rates of hepatic AEs and hepatic AE-related discontinuation of AZA treatment might have been considerably lower in these previous studies. However, observational studies also found a lower frequency of hepatic AEs and a hepatic AE-related AZA treatment discontinuation than in the present study (hepatic AEs occurred in 1.1% of SLE patients and 0.8-4% of IBD patients). Abnormal hepatic laboratory test results were less frequent in these observational studies than in our present study; therefore, the relatively frequent occurrence of abnormal hepatic laboratory test results might be one of the main causes underlying the high discontinuation rate of AZA treatment in the present study. In contrast, the renal function was not related to AEs in the present study. This may be because the initial dose of AZA was adjusted according to the eGFR.

The rates of cytopenic AEs in the present study were comparable to those observed in previous studies: 16-34% in AAV (5, 7, 8), 18-32% in SLE (18, 19, 21), and 7.4-21% in IBD (9-11). However, discontinuation of AZA due to cytopenic AEs was more frequent in the present study than in previous studies: 0-1.3% in AAV (5, 6), 1.9-4.5% in SLE (18, 20), and 8.3% in IBD (9). The initial dose of AZA was lower in the present study (0.63 mg/kg/day) than in a previous study (2 mg/kg/day for maintenance therapy during the first year). The frequent discontinuation of AZA treatment in the present study might be reflective of the fact that the initial AZA dose used was too low to re-administer AZA at an even lower dose to prevent or reduce the occurrence of cytopenic AEs.

Previous studies have shown that immunosuppressants are used only limitedly in Japanese patients with AAV (3, 24). In particular, CYC is not frequently used in Japan for patients with MPA/renal-limited vasculitis (RLV) (24, 25). The relatively low use of AZA in Japan might be associated with this relatively infrequent use of CYC. However, the frequent occurrence of AEs in the present study led to the discontinuation of AZA treatment despite the considerably higher CYC usage in the study cohort. These results suggest that low use of AZA in Japan is associated not only with infrequent use of CYC but also with the frequent occurrence of AEs. A recent clinical trial suggested that rituximab might be used for not only remission induction but also remission maintenance (7). Rituximab may be an alternative to AZA in these patients.

The current study has three limitations. First, a treatment protocol of AZA was not provided, and each attending physician decided whether or not to discontinue the treatment;
thus, AE occurrence might have been overestimated. However, we assessed AEs on the basis of the laboratory data for hepatic and cytopenic AEs. Therefore, the overestimation bias was relatively low, at least for these AEs. Second, we failed to elucidate why 27% of the patients enrolled in the present study exhibited abnormal hepatic laboratory test results at the initiation of the treatment. This issue may be resolved in a larger-scale cohort study of Japanese patients with AAV. Third, each comparison included only a small number of patients, and a multivariate analysis could not be performed, resulting in the possible persistence of confounding factors.

In conclusion, AZA treatment was difficult to continue in approximately 50% of Japanese AAV patients because of AEs. Although the treatment was appropriate for remission maintenance of AAV in tolerable patients, it may not be suitable for patients with abnormal hepatic laboratory test results, low leukocyte counts, or low neutrophil counts.

Author’s disclosure of potential Conflicts of Interest (COI).
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References

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