Liver Injury Risk Factors in Amyotrophic Lateral Sclerosis Patients Treated with Riluzole

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Riluzole, a drug used in the management of amyotrophic lateral sclerosis (ALS), is associated with a high incidence of liver failure. It is imperative to determine risk factors and severity of liver injury in patients taking riluzole to devise an appropriate treatment regimen. We, therefore, studied risk factors for liver injury in ALS patients who were prescribed riluzole at Kitasato University East Hospital from 1999 to 2015. Of the 222 patients enrolled in this study, 113 and 109 patients were diagnosed with mild to moderate (grade 1 or 2) and without (grade 0) liver injury, respectively. Prediction of risk factors was determined using binary logistical regression analyses. The results showed that 50.9% (n = 113) of ALS patients developed mild to moderate liver injury; 71.7% and 53.1% of patients were concurrently using CYP1A2 inhibitors (p = 0.005) and diclofenac (p = 0.032), respectively; 55.8% of patients with liver injury had a history of smoking (p = 0.011). Multivariate analyses revealed that the concurrent use of CYP1A2 inhibitors [odds ratio (OR) 2.152, 95% confidence interval (CI) 1.225–3.780, p = 0.008] and history of smoking (OR 1.938, 95% CI 1.125–3.340, p = 0.017) were independent risk factors for liver injury in patients receiving riluzole. In conclusion, treatment of ALS patients with riluzole, smoking habits, and concurrent use of CYP1A2 inhibitors are independent liver injury risk factors. Further studies on liver injury are warranted in ALS patients treated with riluzole to comprehensively understand the underlying mechanisms of riluzole-associated liver toxicity.

Key words—liver injury; riluzole; risk factor; amyotrophic lateral sclerosis; CYP1A2 inhibitor; smoking habit

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by selective degeneration of the upper and lower motor neurons.1) In Japan, the prevalence of ALS is estimated to be 2–7 cases per 100000 people, while the incidence rate is estimated to be 1–1.5 cases per 100000 people.2) Many patients die within 5 years of diagnosis, indicating that ALS is associated with a poor prognostic outcome.2)

Currently, both riluzole and edaravone are used to treat ALS in Japan. In a systematic review3) on riluzole, based on four clinical trials4–7) that examined survival as the primary endpoint and included 1477 patients (974 riluzole-treated patients and 503 placebo-treated patients), it was concluded that riluzole treatment could prolong the median survival of ALS patients by 2 to 3 months. The Japanese Society of Neurology Practical Guidelines for Amyotrophic Lateral Sclerosis8) recognize riluzole as the only therapeutic drug for ALS treatment and recommend its administration in all ALS patients irrespective of age and degree of disease progression, except in those cases where the vital capacity is less than 60%. Moreover, several clinical studies conducted on edaravone with the revised ALS functional rating scale (ALSFRS-R), which scores ALS symptoms as the primary endpoint, failed to provide significant prognostic outcomes.9,10) Interestingly, the prognostic outcomes were significant within a subset of ALS patients, indicating that edaravone cannot be widely used in all ALS patients.11) As such, riluzole remains the primary drug of choice to treat ALS patients.

A therapeutic trial reported that an increase in the alanine aminotransferase (ALT) levels to more than three times the upper limit of the normal level (ULN) was observed in 7.79% of ALS patients treated with riluzole.4) In another trial, an increase in the ALT levels by up to 3–5 times the ULN level was observed in 7.81% of ALS patients treated with riluzole. The increase in the ALT level was dose-dependent, and this ultimately led to the withdrawal of the treatment modality.5) Symptomatic liver injury was not ob-

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served during these therapeutic trials; however, Remy et al., reported that 2 patients developed acute hepatitis following treatment with riluzole.12

The package insert of riluzole in Japan states that the drug is contraindicated in patients with serious liver injury and should be discontinued in the event of liver injury.13 A previous study has shown that liver function may improve after discontinuation of treatment in cases where liver dysfunction resulted from riluzole usage.4 However, in such cases, treatment should not be resumed due to increased risk of liver re-injury.12

It is imperative to determine the risk factors and severity of liver injury in patients taking riluzole to devise an appropriate treatment regimen. To our knowledge, no previous study has conducted such an investigation. Therefore, this retrospective study aimed to evaluate liver injury risk factors in ALS patients treated with riluzole.

PATIENTS AND METHODS

Study Population Between February 1999 and January 2015, 365 ALS patients who received riluzole at Kitasato University East Hospital, Kanagawa, Japan were initially enrolled (Fig. 1). The exclusion criteria were as follows: 1) any history of disease that could influence liver function test values [chronic hepatitis, autoimmune hepatitis, jaundice, biliary disease, alcohol dependence, various types of viral infection (hepatitis A, hepatitis B, hepatitis C, hepatitis E, hepatitis G, herpes, cytomegalovirus, and Epstein-Barr virus), and multiple organ failure]; 2) diagnosis of liver failure induced by drugs other than riluzole; 3) use of drugs with a higher incidence of liver injury dysfunction than that of riluzole in clinical studies at the time of approval; 4) lack of data on ALT levels in medical records; 5) ALT or aspartate aminotransferase (AST) levels at least 2 times higher than the ULN level, as recommended in a previous study.5

Information on age, sex, height, weight, duration of illness, dosage period, cumulative dose, type of illness, forced vital capacity, history of surgery, transfusion, smoking, allergies, and use of CYP1A2 inhibitors or concomitant drugs that improve liver function (ursodiol, Proheparum®, or Strong neoninophagen C®) was retrospectively collected from patients’ medical records.

It is imperative to be cautious when co-administer-
ing CYP1A2 inhibitors, including diclofenac sodium, caffeine hydrate, theophylline, clomipramine hydrochloride, amitriptyline hydrochloride, imipramine hydrochloride, and fluoroquinolone with riluzole.\(^{13}\) Additionally, fluvoxamine maleate, zafirlukast, methoxsalen, mexiletine hydrochloride, oral contraceptives, acyclovir, allopurinol, cimetidine, and pegylated interferon-α-2a (gene recombination drug) are described in the Drug Interaction Guidelines for Drug Development and Labeling Recommendations.\(^{14}\) In this study, the concurrent use of a drug (CYP1A2 inhibitors and other drugs that improve liver function) implicates prescription use of that drug at least once during the study period, irrespective of dosage and administration.

The present study was conducted in accordance with the Ethical Guidelines for Medical and Health Research Involving Human Subjects issued by the Ministry of Education, Culture, Sports, Science, and Technology and the Ministry of Health, Labour, and Welfare, Japan as well as the Declaration of Helsinki. This study was approved by the Kitasato University Medical Ethics Committee (Section B14-226).

**Definitions** The severity of liver failure was determined based on the maximum ALT level during treatment with riluzole and in accordance with the Common Terminology Criteria for Adverse Events: grade 1 (\(<\)ULN to 3 times the ULN), grade 2 (3 to 5 times the ULN), grade 3 (5 to 20 times the ULN), and grade 4 (\(>\)20 times the ULN).

**Statistical Analysis** Continuous variables are presented using the median and were compared between groups using the Mann-Whitney U test. Categorical variables are presented as frequencies and were compared using Fisher’s exact test. To assess risk factors associated with liver injury, a binomial logistic regression model was constructed. Variables with a p-value of \(<\)0.1 were included in the multivariate analysis. Variables with a p-value of \(<\)0.05 in the multivariate analysis were considered to be significantly associated with liver injury. All analyses on CYP1A2 inhibitors were performed either in combination with all other drugs or individually. Statistical analyses were performed using SPSS\(^\text{®}\) version 24 (IBM Corp., Armonk).

**RESULTS**

A total of 50.9\% (\(n = 113\)) of ALS patients developed mild to moderate liver injury (grade 1, \(n = 104\) and grade 2, \(n = 9\); Tables 1 and 2). Age, sex, height, weight, duration of illness, dosage period, cumulative dose, type of illness, forced vital capacity, history of surgery, transfusion, allergies, and concurrent use of caffeine, amitriptyline, levofoxacin, clomipramine, imipramine, mexiletine, theophylline, fluvoxamine, allopurinol, cimetidine, ursodiol, Proheparum\(^\text{®}\), and Strong neo-minophagen C\(^\text{®}\) showed no difference between patients with mild or moderate liver injury and those with no liver injury.

Among patients with liver injury, 71.7\% and 53.1\% used CYP1A2 inhibitors (\(p = 0.005\)) and diclofenac (\(p = 0.032\)), respectively, and 55.8\% had a history of smoking (\(p = 0.011\)).

**Risk Factors for Liver Injury** Table 3 shows the results obtained from logistic regression analyses. In multivariate analysis, the concurrent use of CYP1A2 inhibitors (OR 2.152, 95% CI 1.225–3.780, \(p = 0.008\)) and history of smoking (OR 1.938, 95% CI 1.125–3.340, \(p = 0.017\)) were independent risk factors for liver injury in ALS patients receiving riluzole (Table 4).

**DISCUSSION**

The exact mechanism of liver toxicity induced by

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**Table 1. General Characteristics of Enrolled Patients**

|                  | Grade 0 (\(n = 109\)) | Grade 1 or 2 (\(n = 113\)) | \(p\)-value |
|------------------|------------------------|-----------------------------|-------------|
| Age (years)      | 66.0 (32.0–92.0)       | 63.0 (37.0–83.0)            | 0.158       |
| Sex (\(n\))     |                         |                             |             |
| Male/female      | 50/59                  | 66/47                       | 0.080       |
| Height (cm)      | 158.0 (136.0–180.0)    | 161.0 (135.5–180.9)         | 0.191       |
| Weight (kg)      | 52.0 (31.0–78.0)       | 54.0 (23.0–92.0)            | 0.172       |
| Duration of illness (d) | 501.0 (14.0–5604.0)   | 532.5 (12.0–3774.0)         | 0.652       |
| Dosage period (d) | 408.5 (7.0–5600.0)    | 461.0 (12.0–3739.0)         | 0.240       |
| Cumulative dose (mg) | 39600.0 (700.0–560000.0) | 48100.0 (1200.0–373900.0) | 0.196       |

Data shown as the median (min–max) and frequency.
Table 2. Clinical Characteristics

|                           | Grade 0 (n = 109) | Grade 1 or 2 (n = 113) | p-value |
|---------------------------|------------------|------------------------|---------|
| Type of illness           |                  |                        |         |
| Bulbar paralysis type     | 26               | 30                     | 0.549   |
| Limb paralysis type       | 81               | 83                     |         |
| Others                    | 1                | 0                      |         |
| Illness type              |                  |                        |         |
| Sporadic                  | 105              | 110                    |         |
| Familial                  | 3                | 3                      |         |
| Not applicable            | 1                | 0                      |         |
| Forced vital capacity     |                  |                        |         |
| ≥ 60%                     | 41               | 39                     | 0.561   |
| < 60%                     | 10               | 7                      |         |
| Not applicable            | 58               | 67                     |         |
| CYP1A2 inhibitor drug     |                  |                        |         |
| Absent                    | 51               | 32                     | 0.005   |
| Present                   | 58               | 81                     |         |
| Surgical history          |                  |                        |         |
| Absent                    | 56               | 56                     | 0.790   |
| Present                   | 53               | 57                     |         |
| Transfusion history       |                  |                        |         |
| Absent                    | 108              | 112                    | 1.000   |
| Present                   | 1                | 1                      |         |
| Smoking history           |                  |                        |         |
| Absent                    | 67               | 50                     | 0.011   |
| Present                   | 42               | 63                     |         |
| Allergies history         |                  |                        |         |
| Absent                    | 89               | 92                     | 1.000   |
| Present                   | 20               | 21                     |         |
| Concurrent use of diclofenac |            |                        |         |
| Absent                    | 67               | 53                     | 0.032   |
| Present                   | 42               | 60                     |         |
| Concurrent use of caffeine |              |                        |         |
| Absent                    | 108              | 112                    | 1.000   |
| Present                   | 1                | 1                      |         |
| Concurrent use of amitriptyline |        |                        |         |
| Absent                    | 104              | 102                    | 0.194   |
| Present                   | 5                | 11                     |         |
| Concurrent use of levofoxacin |           |                        |         |
| Absent                    | 93               | 86                     | 0.091   |
| Present                   | 16               | 27                     |         |
| Concurrent use of clomipramine |         |                        |         |
| Absent                    | 106              | 107                    | 0.499   |
| Present                   | 3                | 6                      |         |
| Concurrent use of imipramine |              |                        |         |
| Absent                    | 109              | 112                    | 1.000   |
| Present                   | 0                | 1                      |         |
| Concurrent use of theophylline |          |                        |         |
| Absent                    | 109              | 112                    | 1.000   |
| Present                   | 0                | 1                      |         |
| Concurrent use of fluvoxamine |           |                        |         |
| Absent                    | 107              | 112                    | 0.617   |
| Present                   | 2                | 1                      |         |
| Concurrent use of allopurinol |            |                        |         |
| Absent                    | 106              | 109                    | 1.000   |
| Present                   | 3                | 4                      |         |
| Concurrent use of cimetidine |           |                        |         |
| Absent                    | 106              | 111                    | 0.679   |
| Present                   | 3                | 2                      |         |
| Concurrent use of ursodiol |              |                        |         |
| Absent                    | 108              | 110                    | 0.622   |
| Present                   | 1                | 3                      |         |

Table 3. Results Obtained from Logistic Regression Analyses

|                                | Odds ratio (lower-upper) | p-value |
|--------------------------------|--------------------------|---------|
| Sex (male/female)              | 0.688 (0.420–1.772)      | 0.688   |
| Smoking history                | 1.784 (0.868–3.669)      | 0.116   |
| Concurrent use of CYP1A2 inhibitor drugs | 1.780 (0.768–4.127) | 0.179   |
| Concurrent use of diclofenac   | 1.146 (0.526–2.495)      | 0.732   |
| Concurrent use of levofoxacin  | 1.445 (0.689–3.033)      | 0.330   |

Table 4. Final Constructed Model following Variable Selection (the multivariate logistic regression)

|                                | Odds ratio (lower-upper) | p-value |
|--------------------------------|--------------------------|---------|
| Constant term                  | 0.469 (0.004)            |         |
| Concurrent use of CYP1A2 inhibitor drugs | 2.152 (1.225–3.780) | 0.008   |
| Smoking history                | 1.938 (1.125–3.340)      | 0.017   |

riluzole remains elusive. An increase in ALT levels is one of the indicators of drug-induced liver injury.15) In our study, 50.9% patients experienced a liver injury, while in a recent retrospective study, an increase in the ALT level of up to double the ULN was observed in 50% of patients.16) Mild to moderate ALT elevation has also been observed in patients with traumatic spinal cord injury undergoing riluzole treatment.17) We found that patients who were using CYP1A2 inhibitors concurrently with riluzole were at higher risk of liver injury. Riluzole is metabolized by CYP1A218) and has been previously reported to cause liver failure in a serum concentration-dependent manner.19) Additionally, van Kan et al. reported a significant positive relationship between riluzole clearance and CYP1A2 activity.20) While some previous reports showed a pharmacokinetic association between riluzole clearance and CYP1A2 activity, no study to date has evaluated liver injury risk factors in ALS patients treated with riluzole. Thus, we believe this is the first study to perform such an analysis. In our study, riluzole metabolism may have been inhibited in patients who were also taking CYP1A2 inhibitors, leading to an elevated blood concentration and increased incidence of liver failure.

Sanderink et al. have evaluated relationships between riluzole and CYP1A2 inhibitors in vitro and reported that riluzole metabolism was inhibited by 99% with clomipramine and diclofenac, 94% with
amitriptyline, 85% with imipramine, 71% with enoxacin, 47% with theophylline, and 37% with caffeine.\(^1\)\(^8\) When CYP1A2 inhibitors were analyzed individually, we found no significant association between any CYP1A2 inhibitors and the development of liver injury. This could be due to the fact that the number of patients analyzed for individual CYP1A2 inhibitors was small, and the period of concurrent use of CYP1A2 inhibitors varied widely, from intake as-needed to daily intake. A prospective study where CYP1A2 inhibitors are concurrently used with other drugs under the same condition is required to investigate the differential influence of individual CYP1A2 inhibitors.

We observed that a history of smoking was an independent risk factor associated with liver injury among patients taking riluzole. Cigarette smoking has been found to induce the expression of CYP1A2 enzymes, which results in accelerated drug clearance.\(^2\)\(^1\)\(^3\) Bruno et al.\(^2\)\(^2\) studied the pharmacokinetics of riluzole in ALS patients and showed that riluzole clearance was lower by 36% in nonsmokers compared with smokers,\(^2\)\(^2\) while Groeneveld et al.\(^2\)\(^3\) suggested that there was no correlation between the serum riluzole concentration and smoking habits.\(^2\)\(^3\) Thus, reports on the association between smoking habits and liver injury due to riluzole are not consistent, and the mechanisms of such an association are unknown. In our study, we could not determine the number of cigarettes consumed and the number of years each patient had been smoking. The blood riluzole concentrations in patients enrolled in this study remained unknown as this was a retrospective study. A prospective study that includes the measurement of blood riluzole concentration is needed to evaluate any association between smoking habits and liver injury resulting from riluzole intake.

This study had several limitations, such as the retrospective study design, unavailability of complete medical records, and the presence of other unknown and unmeasured confounding factors.

In conclusion, ALS patients treated with riluzole, a history of smoking, and concurrent use of CYP1A2 inhibitors are found to be independent liver injury risk factors. Further studies on liver injury are warranted in ALS patients treated with riluzole to comprehensively understand the underlying mechanisms of riluzole-associated liver injury.

**Conflict of Interest** Chihiro Kawano, Yurika Isozaki, Ayumi Nakagawa, Takeshi Hirayama, and Masakazu Kuroyama declare that they have no conflict of interest. Kazutoshi Nishiyama has received grants and personal fees from the Tanabe Mitsubishi Pharmaceutical Company for work unrelated to this study.

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