

# A Randomized, Single-Blind, Placebo-Controlled, 3-Way Crossover Study to Evaluate the Effect of Therapeutic and Supratherapeutic Doses of Edaravone on QT/QTc Interval in Healthy Subjects

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## Abstract

This randomized, single-blind, 3-way crossover study assessed the effect of edaravone on QT interval, including an exposure-response analysis. Twenty-seven healthy Japanese male volunteers, aged 20 to 49 years, were randomly assigned to receive a single intravenous dose of each treatment in 1 of 3 sequences ( $n = 9$  each): ACB, BAC, and CBA, where A was edaravone 60 mg (therapeutic dose), B was edaravone 300 mg (supratherapeutic dose), and C was normal saline (placebo). Electrocardiographs were collected to assess treatment effects. In an exposure-response analysis, a linear model was determined to be valid and indicated no statistically significant positive slope for the relationship between change from baseline in QTcF ( $\Delta$ QTcF) and edaravone concentration ( $0.000155 \text{ ms}/(\text{ng}/\text{mL})$ ;  $P = .1478$ ); upper bounds of 2-sided 90% confidence intervals after placebo adjustment ( $\Delta\Delta$ QTcF) were  $<10$  milliseconds at the geometric mean maximum concentration for each edaravone dose. Overall estimated values by time point of  $\Delta\Delta$ QTcF  $\leq 0.9$  milliseconds, no outlier values, and no morphologic changes suggestive of repolarization abnormalities were observed. Analysis of heart rate, PR interval, and QRS duration also revealed no adverse findings. These data indicate that edaravone, even at supratherapeutic doses, does not produce clinically meaningful QT prolongation and has no clinically relevant cardiac effects.

## Keywords

cardiovascular safety, concentration-QTc model, edaravone, ICH E14, QTc interval

The small molecule edaravone (MCI-186; Mitsubishi Tanabe Pharma Corporation, Tokyo, Japan) is a free-radical scavenger developed as a neuroprotectant. Its mechanism of neuroprotective activity is inhibition of phospholipid-membrane degradation by free radicals and, therefore, protection against damage from oxidative stress.<sup>1-4</sup> Edaravone was first approved for the treatment of acute ischemic stroke in 2001 in Japan.<sup>5</sup> It is also approved for the treatment of amyotrophic lateral sclerosis in Canada, China, Japan, South Korea, Switzerland, and the United States.<sup>6,7</sup>

In human and animal studies, pharmacokinetic (PK) measurements such as peak plasma edaravone concentration ( $C_{\text{max}}$ ) and area under the plasma concentration–time curve (AUC) were found to in-

crease as the dose increased.<sup>8-11</sup> The principal route of excretion of edaravone and of its glucuronide and

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Mitsubishi Tanabe Pharma Corporation, Tokyo, Japan

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sulfate metabolites is urinary,<sup>8-10</sup> and the amount of unchanged edaravone excreted to urine is approximately 1% of the administered dose.<sup>8</sup> Edaravone is highly protein-bound (91%–92%) and is not accumulated after repeated administrations.<sup>12</sup> Neither edaravone nor its metabolites inhibit cytochrome P450 isozymes, uridine diphosphate glucuronosyltransferases, or transporters, nor do they induce cytochrome P450, when administered at the recommended dose.<sup>12</sup> Moreover, no differences in edaravone PK profiles according to ethnicity, sex, age, or weight were reported from a population PK analysis of pooled data from phase 1 studies in healthy participants.<sup>8</sup>

From early in clinical development, all drugs undergo clinical electrocardiographic evaluation, which generally includes a single trial focused on assessing each drug's effect on cardiac repolarization in healthy volunteers.<sup>13</sup> Such a study is designed to determine whether a drug has a threshold pharmacologic effect on cardiac repolarization, as detected by QT/QTc prolongation (around 5 milliseconds, as evidenced by an upper bound of the 95% confidence interval [CI] around the mean effect on QTc of 10 milliseconds).<sup>13</sup>

Exposure-response analysis can be used as the primary end point for a cardiac repolarization study: Such analysis consists of regression modeling of placebo-adjusted changes in QTc from baseline, as a function of concentration for all dose levels combined. A suprathreshold drug dose must be included; that is, the concentration level to be attained is based on the highest concentration anticipated during normal clinical use (high clinical exposure scenario; ie, if adverse alterations in metabolism or elimination occur at the therapeutic dose).<sup>14</sup>

An *in vitro* manual patch clamp study at body temperature in human embryonic kidney-293 transfected cells showed no effect (<5.0% inhibition) for up to 100  $\mu$ M of edaravone on the human-ether-à-go-go-mediated cardiac potassium ion current.<sup>15</sup> These results suggest at least a 100-fold margin of safety for an anticipated therapeutic dose of edaravone 60 mg over 60 minutes for the treatment of amyotrophic lateral sclerosis. In an earlier phase 1 study in 46 healthy volunteers, an initial bolus of edaravone was followed by continuous infusions over 24 hours, with total doses up to 12.0 mg/kg; mean  $C_{max}$  in the highest-dose group was 1164 ng/mL. In this group, least squares mean placebo-adjusted changes in QTc interval (using Fridericia's formula [QTcF]<sup>16</sup>) from baseline were between -10.3 and +5.9 milliseconds during 48 hours of observation; no statistically significant differences were evident between the edaravone and placebo groups.<sup>17</sup> However, it was considered that this phase 1 study had several limitations that made the study uninterpretable for excluding small QTc effects

(10-millisecond threshold).<sup>13,14</sup> No suprathreshold dose/exposure was studied; therefore, QTc effects at the high clinical exposure scenario were not characterized; electrocardiographic assay sensitivity was not established because there was no positive control or any higher dose; and electrocardiogram (ECG) quality and electrocardiographic/PK assessments were inadequate because of single ECGs (not replicates), and inadequate matched electrocardiographic/PK sampling points led to difficulties in excluding possible hysteresis.

A postmarketing "thorough QT/QTc" study of edaravone was considered necessary to exclude small QT prolongation effects (10-millisecond threshold). Thus, the objective of the current trial was to evaluate (by exposure-response analysis) the effect of therapeutic and suprathreshold doses of edaravone on the QT interval (corrected for heart rate [HR] using QTcF<sup>16</sup>) in healthy volunteers.

## Materials and Methods

### Study Design

This was a phase 1, randomized, single-blind, placebo-controlled, 3-way crossover, single-center study. The study was designed according to standards set forth in the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use E14 (including the Q&A [R3] update in December 2015)<sup>12,13</sup> to include PK data and to designate exposure-response analysis as the method for primary analysis.

Pharmacodynamic (PD) ECGs paired with time-matched PK determinations were analyzed. The doses administered were treatment A (therapeutic dose of edaravone): a single intravenous (IV) dose of edaravone 60 mg administered over 60 minutes; treatment B (suprathreshold dose of edaravone): a single IV dose of edaravone 300 mg administered over 60 minutes; and treatment C (placebo): a single IV dose of normal saline (0.9% w/v) administered over 60 minutes. ECGs were collected to assess the effects of each treatment.

Suprathreshold doses of edaravone were used to assess the response at a sufficiently high multiple of exposure levels to cover the highest concentration anticipated during normal clinical use, according to ICH E14 guidance and a recent scientific white paper.<sup>13,14,18</sup> Thus, a positive control was not considered necessary in our study.

### Study Ethics

The study was conducted at Souseikai Sumida Hospital, Tokyo, Japan. The locally appointed institutional review board at Souseikai Sumida Hospital approved the research protocol, and the study was conducted in accordance with ethical principles outlined in the

Declaration of Helsinki and consistent with Good Clinical Practice (GCP), as also described in ICH Harmonised Tripartite Guidelines for GCP 1996, Japan-GCP, and the Law for Ensuring the Quality, Efficacy, and Safety of Drugs and Medical Devices (Trial registration: NCT04029090). The study was also conducted in accordance with all regional and local legal requirements. All subjects completed an informed consent form and provided written informed consent to participate in the study.

### Subject Eligibility

The study was planned to enroll 27 healthy male volunteers, aged 20 to 55 years. Nine volunteers were to be randomized to each of 3 treatment sequences: ACB, BAC, and CBA.

**Inclusion Criteria.** All subjects had a body weight  $\geq 45$  kg and body mass index 18 to 30 kg/m<sup>2</sup> at screening and day -1. All subjects were in good general health and free from clinically significant illness or disease in the opinion of the investigator.

**Exclusion Criteria.** The principal exclusion criteria comprised (at screening or day -1): pulse rate  $>240$  milliseconds, QRS  $\geq 120$  milliseconds, QTcF  $>450$  milliseconds, or any clinically significant electrocardiographic abnormality; a history of cardiac disease or arrhythmias likely to cause QTc prolongation; a family history of torsade de pointes, long-QT syndrome, hypokalemia, or sudden death; potassium levels outside laboratory reference ranges; clinically significant deviations from normal (in the opinion of the investigator) in physical examination, vital signs, ECG, or clinical laboratory tests; the presence or history of any clinically significant disease or organ dysfunction in the opinion of the investigator. Other exclusion criteria are listed in the Supplemental Information.

### Study Endpoints

The main study endpoint was based on the regression relationship between change from baseline in QTcF ( $\Delta$ QTcF), after placebo adjustment ( $\Delta\Delta$ QTcF), and concentration of edaravone for all time points and treatments combined. Additional endpoints comprised: assessment of HR, PR interval, QRS interval, and QTcF by time point; categoric outliers for QTcF interval (absolute value and change from baseline) and other 12-lead ECG parameters; incidence of abnormalities in ECG morphology; PK parameters for edaravone; the incidence of adverse events (AEs) and serious AEs; vital signs; safety; 12-lead ECG variables; and results of laboratory tests and physical examination.

### Electrocardiographic Assessment

Continuous 12-lead Holter data were obtained from all subjects from  $\geq 1$  hour before until  $\geq 24$  hours

after starting the edaravone infusion. An experienced cardiology core laboratory provided centralized and standardized interpretation of the ECGs. Technically optimal ECGs from the continuous Holter data were extracted and interpreted via manual adjudication of semi-automated interval determination in a digital environment. A small number of expert medical electrocardiographers were assigned, and a single reader reviewed all ECGs from any individual subject under blinded conditions.

Triplicate 10-second, 12-lead PD ECGs (taken approximately 1 minute apart) were extracted from the 5-minute period starting at the following time points: 0.75, 0.50, and 0.25 hours before the infusion; and at 0.5, 1, 1.25, 1.5, 1.75, 2, 3, 4, 6, 8, 12, and 24 hours after starting the infusion on day 1. Before each of these time points, subjects had to rest for  $\geq 10$  minutes, and rest continued for 5 minutes after each time point. The electrocardiographic parameters studied were HR, corrected QT interval, PR interval, and QRS duration. The formula used for all analyses of corrected QT data was the Fridericia correction:  $QTcF = QT/RR^{(1/3)}$ , which assumes RR to be in seconds. Within a set of triplicate ECG measurements at each time point, each individual QT and RR value was used to calculate a QTcF interval. These individual QTcF intervals across triplicates were then averaged for analysis. Other ECG parameters also represented the mean of triplicate parameters for each subject at each time point, based on ECGs extracted at each time point from Holter data. When triplicate ECGs could not be extracted, the mean of 2 ECGs or a single ECG value was used. Overall, failure to extract triplicate ECGs occurred infrequently; there were just 5 samples from which triplicate ECGs could not be extracted, 3 of which were single ECGs.

### Pharmacokinetic Assessments

Blood samples for edaravone were collected before dosing and at 0.5, 1, 1.25, 1.5, 1.75, 2, 3, 4, 6, 8, 12, and 24 hours after the start of the infusion. Blood samples were collected 5 to 7 minutes after the time points at 0.5, 1, 1.25, 1.5, and 1.75 hours after infusion start, and 5 to 10 minutes after the time points at 2, 3, 4, 6, 8, 12, and 24 hours after infusion start due to ECG monitoring at the same time points. Blood tubes were kept on ice prior to centrifugation (4°C, 1500  $\times$  g, 10 minutes). The resultant plasma samples (500  $\mu$ L) were combined with an internal standard (<sup>13</sup>C-edaravone), stabilizer (4'-Me-edaravone) and McIlvaine buffer (pH 5.4) and transferred to polypropylene tubes and stored (within 2 hours after sampling) at -20°C or lower to await assay.

Plasma drug concentrations were determined using validated liquid chromatography-mass spectrometry methods. Following addition of 5 mL of extraction solvent (dichloromethane/pentane [30:70, v/v]), the mix

was shaken for 10 minutes and centrifuged (4°C, 1900 × *g*, 5 minutes). The organic layer was evaporated to dryness under nitrogen stream at 45°C, and the injection sample was prepared by reconstituting the residue. Chromatographic separation was achieved using Symmetry Shield RP18 (100 × 4.6 mm, 3.5 μm; Waters Corp., Milford, Massachusetts) with the isocratic condition of water/formic acid (1000:1, v/v) and methanol (2:3) at a 0.5 mL/min flow rate. A triple-quadrupole mass spectrometer (API5000, AB SCIEX Pte. Ltd., Framingham, Massachusetts) in multiple reaction monitoring mode was used to detect the analyte. Monitored ion ratios were *m/z* 175 → *m/z* 106 for edaravone and *m/z* 179 → *m/z* 107 for the internal standard. Peak areas for both analyte and the internal standard were determined using Analyst 1.6.2 (AB SCIEX Pte. Ltd.). The analytic range for quantification of edaravone was 1 to 1000 ng/mL. Values below the lower limit of quantification were treated as 0. Acceptance criteria for accuracy were within ±20.0% (lower limit of quantification) and within ±15.0% (other concentrations); all calibration and/or quality control samples met these criteria. Confirmation of sensitivity was performed by injection of a standard in each analytical run, prior to sample injection; no inappropriate results (in analyte and internal standard retention times, peak shapes, or equipment sensitivity) were observed. All PK parameters were estimated using Phoenix WinNonlin (version 6.3; Certara Inc., Princeton, New Jersey) by noncompartmental analysis and 3-compartment model analysis. The PK parameters evaluated included  $C_{\max}$ , area under the plasma concentration–time curve from time zero to infinity ( $AUC_{0-\infty}$ ), and half-life at the terminal elimination phase. Summary statistics of PK parameters (geometric mean or mean with percent coefficient of variation) were calculated. Plasma concentration data at each time point were summarized, and plasma concentration–time profiles of edaravone were plotted.

### Safety Assessment

Safety was evaluated throughout the study by monitoring AEs, vital signs, 12-lead ECG variables, and laboratory test and physical examination results. Vital signs (blood pressure, pulse rate, and body temperature), routine safety 12-lead ECG using conventional bedside equipment, and physical examinations were conducted at screening, day –1, 1 hour before dosing, 1 hour and 24 hours after the start of the infusion, and at follow-up; in addition, laboratory tests (hematology, biochemistry, coagulation, and urinalysis) were conducted at screening, day –1, 24 hours after the start of the infusion, and at follow-up. Treatment-emergent AEs (TEAEs) were classified using the Medical Dictionary for Regulatory Activities (version 20.0) and summarized using system organ class and preferred term.

### Sample Size

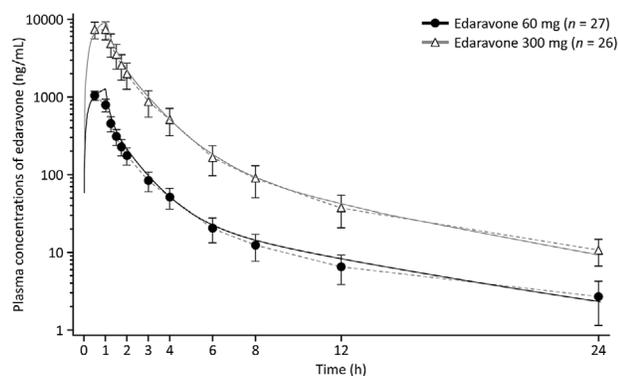
The projected sample size of 27 subjects was not based on a formal power calculation. However, based on a relevant publication,<sup>19</sup> even if assuming a maximum  $\Delta\Delta QTcF$  of 5 milliseconds at the suprathreshold dose, 24 subjects at each dose or placebo would be adequate to exclude the upper bound of the 90%CI of 10 milliseconds with power ≥80%. In addition, 24 subjects would provide ≥90% power if the upper bound of the 90%CI was <10 milliseconds, assuming a  $\Delta\Delta QTcF$  of 0 milliseconds at edaravone  $C_{\max}$  after a 60-mg or 300-mg dose and based on simulation using previous studies.<sup>17</sup> Thus, to allow for potential study dropouts, a total of 27 subjects were randomly assigned to treatment in this study.

### Statistical Analysis

Statistical analysis was performed using SAS version 9.4 (SAS Institute, Cary, North Carolina). All cardiac analyses involved all randomized subjects who received at least 1 dose of edaravone and who had at least one 12-lead PD ECG measurement after dosing. Edaravone concentration assessment data were combined with PD ECG values for the cardiac analyses.

The primary regression analysis was performed between  $\Delta QTcF$  values at each time point and the matching edaravone concentration for both edaravone dose levels combined.  $\Delta QTcF$  was the subject-specific value of change from baseline in  $QTcF$ . A linear mixed-effects model was created with  $\Delta QTcF$  as the dependent variable, edaravone plasma concentration and adjusted baseline  $QTcF$  (subjects' baseline per period minus mean baseline for all subjects per period) as continuous covariates, time (hours) and treatment as categorical factors, and a random intercept and slope per subject. This model failed to converge. For thorough QTc crossover study designs, a subject-level and period-level random intercept specification rather than a subject-level random intercept and slope specification are considered to have a better model fit.<sup>20</sup> Thus, the final model employed a random intercept per subject and per period. If upper bounds of the 2-sided 90%CIs of point estimates for placebo-adjusted  $\Delta QTcF$  ( $\Delta\Delta QTcF$ ) at each geometric mean  $C_{\max}$  of edaravone (for each edaravone dose level) were <10 milliseconds, then no clinically meaningful QTc interval prolongation was concluded.

In an exploration to determine the appropriate model, model assumptions were assessed to show that there was an insignificant effect of edaravone on change of HR, and that there was adequate normalization of  $QTcF$  for RR. Hysteresis was also examined. A delay of >1.0 hours for mean  $\Delta\Delta QTcF$  with respect to  $C_{\max}$  for the edaravone suprathreshold dose group was considered to indicate hysteresis,



**Figure 1.** Profile of mean observed (plot with standard deviation error bars and dashed line) and mean predicted (solid line) plasma concentrations (compartment model analysis; semilogarithmic plots).

unless all estimated  $\Delta\Delta\text{QTcF}$  values were  $<5$  milliseconds. Choices of linear-model and goodness-of-fit plots were assessed to confirm model validity.<sup>18</sup> The goodness-of-fit plots evaluated included residuals vs edaravone concentration, baseline QTcF, treatment, or time point; model-predicted  $\Delta\text{QTcF}$  vs observed  $\Delta\text{QTcF}$ ; quantile-quantile plot of residuals; and quantiles of concentration and  $\Delta\text{QTcF}$  overlaid with the slope of the final model. Quantile plots of observed data overlaid with the model prediction produced for each time point from the analysis mentioned above and as the goodness-of-fit plot for another similar concentration-QTcF analysis with  $\Delta\Delta\text{QTcF}$  as the dependent variable were also generated.

As an additional analysis, an intersection-union test (whether or not the 1-sided 95%CI upper bound was lower than 10 milliseconds at all time points) of  $\Delta\Delta\text{QTcF}$  was analyzed using a mixed model for repeated measures. The final model included treatment, scheduled visits, period, and sequence as fixed effects, corresponding baseline-derived parameters and ECG as covariates, and treatment-by-visit and baseline-by-visit interactions. An unstructured correlation matrix was used to model within-subject and within-period variance-covariance errors. From this model, placebo-adjusted least squares mean  $\Delta\text{QTcF}$  (estimated  $\Delta\Delta\text{QTcF}$ ) and the 1-sided 95%CI upper bound were presented. This analysis was also used for the examination of hysteresis. An identical concentration regression analysis was performed for HR, PR interval, and QRS duration, and an identical by-time-point analysis was also performed for HR, PR interval, and QRS duration.

Other assessments were mean QTcF; the proportion of subjects with a QTcF interval  $>450$ ,  $>480$ , and  $>500$  milliseconds; the proportion of subjects with an increase from baseline in QTcF interval of  $>30$  and  $>60$  milliseconds; the proportion of subjects with

HR, PR interval, or QRS duration abnormalities (ie, HR  $<40$  beats per min [bpm] after a decrease in HR of  $\geq 20$  bpm from baseline, or HR  $>110$  bpm after an increase in HR of  $\geq 20$  bpm from baseline; PR interval  $<100$  milliseconds after a decrease in PR interval of  $\geq 25\%$  from baseline, or PR interval of  $>220$  milliseconds after an increase in PR interval of  $\geq 25\%$  from baseline; or change of QRS duration  $\geq 25\%$  from baseline reaching a QRS  $\geq 120$  milliseconds); and the proportion of subjects with an emergent ECG diagnostic morphologic abnormality.

## Results

### Subject Characteristics

Overall, 27 Japanese healthy male subjects were enrolled and treated. Median age was 27 (range, 20–49) years, and median body mass index was 21.84 (range, 18.0–25.0)  $\text{kg}/\text{m}^2$  (Table 1). All subjects received edaravone 60 mg IV ( $n = 27$ ), all but 1 received edaravone 300 mg IV ( $n = 26$ ), and all but 2 received placebo ( $n = 25$ ). One subject in the ACB sequence group discontinued from the study on day  $-1$  of the second treatment due to a protocol violation, and did not receive placebo or edaravone 300 mg; 1 subject in the BAC sequence group withdrew consent and discontinued from the study on day  $-1$  of the third treatment and did not receive placebo.

**Pharmacokinetics.** The observed profile of edaravone plasma concentrations with simulated curves resulting from 3-compartment model analysis is shown in Figure 1. Descriptive statistics for PK parameters of unchanged edaravone calculated using noncompartmental analysis and 3-compartment model analysis are summarized in Table 2.  $C_{\text{max}}$  values for the edaravone 60-mg and 300-mg doses were 1030 and 7566 ng/mL, respectively; corresponding values for  $\text{AUC}_{0-\infty}$  were 1549 and 12920  $\text{ng}\cdot\text{h}/\text{mL}$  from the noncompartmental analysis. With the higher edaravone dose, increases in  $C_{\text{max}}$  and  $\text{AUC}_{0-\infty}$  were slightly higher than the dose ratio.

**Regression Analysis.** For the QTcF-concentration regression analysis, a linear model was found to best fit the data among linear, quadratic, and cubic models. For the linear model, linear slope was 0.000155  $\text{ms}/(\text{ng}/\text{mL})$  ( $P = .1478$ ), which was not significantly different from a slope of 0. The 2-sided slope 90%CI ranged from  $-0.000021$  to 0.000332  $\text{ms}/(\text{ng}/\text{mL})$  (Table 3).

Estimated mean values of  $\Delta\Delta\text{QTcF}$  at geometric mean  $C_{\text{max}}$  levels for the edaravone doses had CI upper bounds  $<10$  milliseconds (Table 3). At the maximum geometric mean  $C_{\text{max}}$  for edaravone 300 mg, 7566 ng/mL, the estimated  $\Delta\Delta\text{QTcF}$  was 0.5 milliseconds with a 2-sided 90%CI upper bound of 1.9 milliseconds. At the geometric mean  $C_{\text{max}}$  for

**Table 1.** Subject Characteristics According to Treatment Sequence

Characteristics, Unit		ACB (n = 9)	BAC (n = 9)	CBA (n = 9)	Total (N = 27)
Age, y	Mean (SD)	29.3 (9.8)	28.7 (10.6)	33.6 (9.8)	30.5 (9.9)
	Median (range)	26.0 (20–49)	24.0 (20–49)	31.0 (21–47)	27.0 (20–49)
Height, cm	Mean (SD)	169.50 (4.53)	172.71 (5.30)	172.61 (7.28)	171.61 (5.79)
	Median (range)	168.90 (162.1–175.1)	172.50 (164.7–179.3)	172.30 (161.9–181.6)	171.50 (161.9–181.6)
Body weight, kg	Mean (SD)	64.48 (4.71)	65.09 (8.35)	62.91 (9.03)	64.16 (7.36)
	Median (range)	64.40 (58.0–72.5)	64.90 (53.6–80.3)	62.10 (51.4–77.5)	64.40 (51.4–80.3)
BMI, kg/m <sup>2</sup>	Mean (SD)	22.44 (1.37)	21.77 (2.04)	21.01 (1.63)	21.74 (1.74)
	Median (range)	21.92 (20.9–24.7)	21.84 (18.0–25.0)	20.92 (18.6–23.5)	21.84 (18.0–25.0)

BMI, body mass index; SD, standard deviation.

A = edaravone 60 mg, B = edaravone 300 mg, C = placebo.

**Table 2.** Plasma PK Parameters of Unchanged Edaravone (Observed [Noncompartmental Analysis] and Predicted by the 3-Compartment Model Analysis)

Parameter, Unit	Noncompartmental Analysis		3-Compartment Model Analysis	
	Edaravone 60 mg (n = 27)	Edaravone 300 mg (n = 26)	Edaravone 60 mg (n = 27)	Edaravone 300 mg (n = 26)
$C_{max}$ , ng/mL	1039 (13.5) <sup>a</sup> 1030 (14.1) <sup>b</sup>	7781 (23.5) <sup>a</sup> 7566 (25.1) <sup>b</sup>	1195 (14.2) <sup>a</sup>	9245 (24.4) <sup>a</sup>
$AUC_{0-\infty}$ , ng · h/mL	1581 (20.8) <sup>a</sup> 1549 (20.5) <sup>b</sup>	13530 (29.4) <sup>a</sup> 12920 (33.3) <sup>b</sup>	1738 (19.0) <sup>a</sup>	14180 (28.0) <sup>a</sup>
$t_{1/2}$ , h	9.19 (75.0) <sup>a</sup>	6.05 (11.1) <sup>a</sup>	7.39 (85.8) <sup>a</sup>	6.22 (35.9) <sup>a</sup>

$AUC_{0-\infty}$ , area under the concentration-time curve from time 0 to infinity;  $C_{max}$ , peak plasma concentration; CV, coefficient of variation; PK, pharmacokinetic;  $t_{1/2}$ , half-life at the terminal elimination phase.

<sup>a</sup>Mean (CV%).

<sup>b</sup>Geometric mean (geometric CV%).

edaravone 60 mg, 1030 ng/mL, the estimated  $\Delta\Delta QTcF$  was  $-0.5$  milliseconds with a 2-sided 90%CI upper bound of 0.5 milliseconds.

Regarding the effect of HR on the model, change of HR from baseline ( $\Delta HR$ ) as a function of concentration had a very small slope of  $-0.000138$  bpm/(ng/mL) without significant difference from a slope of 0 ( $P = .1034$ ). Two-sided 90%CIs of placebo-adjusted values ( $\Delta\Delta HR$ ) were 1.2 bpm to  $-3.1$  bpm across the full range of concentrations, a clinically insignificant effect. Thus, it was concluded that there was an insignificant effect of edaravone on change of HR and no HR influence on the model.

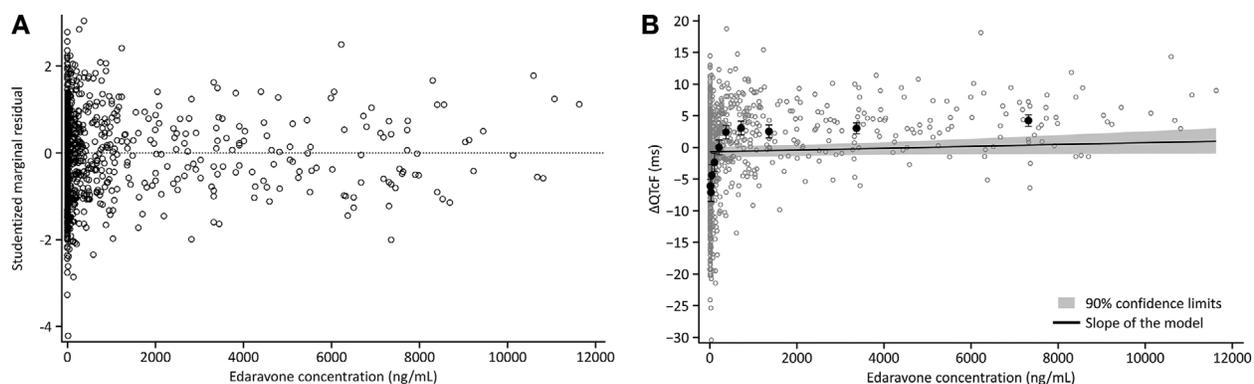
Regarding the effect of QT correction, the QTcF vs RR regression had a positive slope (0.038583), which was statistically significantly different from a slope of 0 ( $P < .0001$ ), but was of no clinical consequence for interpretation of QTcF. By observation, over the wide range of RRs from 650 to 1600 milliseconds, there was a change of  $\pm 15$  milliseconds from the overall mean QTcF (from about 390 to 420 milliseconds).

For the examination of hysteresis, the maximum estimated  $\Delta\Delta QTcF$  by time point increase was at 60 minutes after the start of infusion (0.9 milliseconds, edaravone 300 mg), and no estimated  $\Delta\Delta QTcF$  values were  $\geq 5$  milliseconds. Thus, there was no effect of hysteresis on validity of the model. The model was supported by goodness-of-fit plots, which showed residuals scattered randomly around 0 (for residual vs edaravone concentration, baseline QTcF, treatment, or time point) or residuals on the line of unity (for model predicted versus observed  $\Delta QTcF$  and quantile-quantile plot of residuals). The plot of residuals vs edaravone concentration is shown in Figure 2A, and the quantile plot of concentration and  $\Delta QTcF$  overlaid with the slope of the final model are shown in Figure 2B. Figure S1 shows the quantile plot of concentration and  $\Delta QTcF$  overlaid with the predicted slope produced for each time point, and Figure S2 shows the quantile plot of concentration and  $\Delta\Delta QTcF$  overlaid with the slope predicted by the model analysis with  $\Delta\Delta QTcF$  as the dependent variable.

**Table 3.** Edaravone QTcF Regression Analysis: Estimated Mean Placebo-Adjusted Change of QTcF From Baseline ( $\Delta\Delta$ QTcF) at Various Edaravone Concentration Levels and 2-Sided 90%CBs (ms)

Concentration Level	Concentration (ng/mL)	Mean $\Delta\Delta$ QTcF (ms)	2-Sided Lower 90%CB (ms)	2-Sided Upper 90%CB (ms)
By quintiles of concentration for all doses				
Minimum	1	-0.7	-1.7	0.3
First quintile	22	-0.7	-1.7	0.3
Second quintile	141	-0.7	-1.7	0.3
Third quintile	484	-0.6	-1.6	0.4
Fourth quintile	2075	-0.4	-1.4	0.6
Maximum	11630	1.1	-1.0	3.2
By geometric mean of individual observed $C_{max}$				
$C_{max}$ edaravone at 60 mg	1030	-0.5	-1.5	0.5
$C_{max}$ edaravone at 300 mg	7566	0.5	-1.0	1.9

CB, confidence bound;  $C_{max}$ , peak plasma concentration.



**Figure 2.** Goodness-of-fit plots for edaravone QTcF-concentration regression analysis. A, Concentration versus residuals. B, Quintiles of concentration and change from baseline in QTcF ( $\Delta$ QTcF) overlaid with slope of final model and individual data; open circles indicate individual values, each closed circle indicates mean QTcF vs median edaravone concentration (for each quintile group) and bars indicate 90% confidence intervals. Studentized marginal residual is the quotient resulting from the division of a residual by an estimate of its standard deviation; this technique is a form of the Student's *t*-statistic and is commonly used to compare residuals at different data points in regression analyses where the standard deviations of residuals in a sample may vary greatly from one data point to another.

### Additional Endpoints

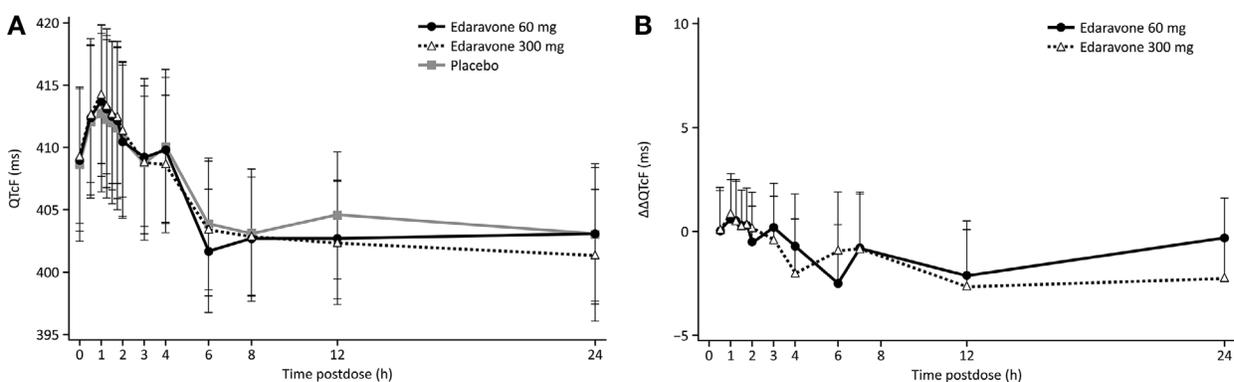
Mean QTcF values were normal and mean changes from baseline included both increases and decreases. There tended to be greater decreases at later time points. Findings for the edaravone groups were similar to those for placebo (Figure 3A). The maximum mean increase from baseline  $\pm$  standard deviation was  $5.0 \pm 5.23$  milliseconds (2-sided 90%CI: 3.2–6.7 milliseconds) for the edaravone 300-mg group at 60 minutes after the start of the infusion.

Determination of the estimated  $\Delta\Delta$ QTcF at each time point by dose showed all values of the one-sided 95%CI upper bounds to be <10 milliseconds. The overall maximum value of estimated  $\Delta\Delta$ QTcF was 0.9 milliseconds for edaravone 300 mg at 60 minutes after the start of the infusion; the maximum among

the upper bounds of the 95%CI was 2.8 milliseconds (Figure 3B).

One subject had several QTcF values >450 milliseconds both with edaravone 60-mg dosing and with placebo; however, this subject had a baseline QTcF value of 453.9 milliseconds before edaravone 60-mg IV dosing. There were no subjects with values of QTcF >480 milliseconds and no values of  $\Delta$ QTcF were >30 milliseconds. Therefore, no clinically relevant QTcF outlier values were found.

No subject had emergent abnormal diagnostic findings relating to morphology, specifically no emergent findings of abnormal ST segments, T waves, or U waves. The relationship of change in PR interval from baseline ( $\Delta$ PR) and change of QRS from baseline ( $\Delta$ QRS) were without significant slopes, as was the relationship



**Figure 3.** A, Arithmetic mean QTcF and 2-sided 90% confidence intervals (ms). B, Mean placebo-adjusted change from baseline in QTcF ( $\Delta\Delta$ QTcF) and 1-sided upper 95% confidence intervals (ms).

**Table 4.** Edaravone Regression Analysis: Estimated Mean Placebo-Adjusted Changes in Heart Rate (HR) and PR and QRS Intervals From Baseline at Various Edaravone Concentration Levels and 2-Sided 90% CBs (ms)

Concentration Level	Concentration (ng/mL)	Mean $\Delta\Delta$ HR (90%CB) (bpm)	Mean $\Delta\Delta$ PR (90%CB) (ms)	Mean $\Delta\Delta$ QRS (90%CB) (ms)
By quintiles of concentration for all doses				
Minimum	1	0.2 (−0.8-1.2)	−0.6 (−2.4-1.1)	0.2 (−0.8-1.1)
First quintile	22	0.2 (−0.8-1.2)	−0.6 (−2.4-1.1)	0.1 (−0.8-1.1)
Second quintile	141	0.2 (−0.8-1.2)	−0.6 (−2.3-1.1)	0.1 (−0.8-1.0)
Third quintile	484	0.1 (−0.9-1.2)	−0.5 (−2.2-1.2)	0.1 (−0.8-1.0)
Fourth quintile	2075	−0.1 (−1.1-0.9)	0.1 (−1.6-1.8)	−0.1 (−1.0-0.8)
Maximum	11630	−1.4 (−3.1-0.3)	3.7 (0.9-6.4)	−1.5 (−3.0-0.0)
By geometric mean of individual observed $C_{max}$				
$C_{max}$ edaravone at 60 mg	1030	0.1 (−0.9-1.1)	−0.3 (−2.0-1.4)	0.0 (−0.9-0.9)
$C_{max}$ edaravone at 300 mg	7566	−0.8 (−2.2-0.5)	2.2 (0.0-4.3)	−0.9 (−2.1-0.2)

bpm, beats per minute; CB, confidence bound;  $C_{max}$ , peak plasma concentration.

of change in HR from baseline ( $\Delta$ HR). Placebo-adjusted values ( $\Delta\Delta$ HR,  $\Delta\Delta$ PR, and  $\Delta\Delta$ QRS) and their 2-sided 90% CIs at the geometric mean  $C_{max}$  values for the 2 treatments were minimal (Table 4). Mean values for HR, PR, and QRS intervals were within normal ranges, and values were similar between the 2 edaravone groups and placebo. There were no outlier values for HR (<40 or >110 bpm, or  $\Delta$ HR  $\pm$  20 bpm reaching those levels), PR interval (<100 milliseconds or >220 milliseconds after an increase or decrease of  $\geq$ 25% reaching that level), or QRS interval (>120 milliseconds, or an increase of  $\geq$ 25% reaching that level).

**Safety.** A total of 3 TEAEs were reported in 3 subjects. No TEAEs led to treatment discontinuation. Two TEAEs (pharyngitis in the edaravone 60-mg group and headache in the placebo group) were mild in severity, and 1 (gastroenteritis in the edaravone 300-mg group) was moderate in severity. All TEAEs resolved, and none of the TEAEs were considered treatment related. No serious AEs, including death, were reported. There were no clinically significant findings or treatment-related

trends regarding vital signs, 12-lead ECG variables, laboratory tests, and physical examinations.

## Discussion

This study assessed the effect of edaravone 60 mg IV (therapeutic dose) and edaravone 300 mg IV (supratherapeutic dose) on cardiac repolarization in healthy volunteers. QTcF interval was the variable for the main regression analysis, and placebo was included as a control. A linear model was determined to be valid and indicated that there was no statistically significant positive slope of the relationship between  $\Delta$ QTcF and edaravone concentration (0.000155 ms/(ng/mL);  $P = .1478$ ); upper bounds of the 2-sided 90% CIs (equivalent to 1-sided 95% upper confidence bounds) of  $\Delta\Delta$ QTcF were all <10 milliseconds at the geometric mean  $C_{max}$  for each edaravone dose level. Thus, the main outcome measure indicated that edaravone had no clinically relevant effect on QT prolongation, as defined by ICH E14 guidance.<sup>13</sup> Additional analyses found estimated values of  $\Delta\Delta$ QTcF  $\leq$ 0.9 milliseconds,

no outlier values, and no morphologic changes suggestive of repolarization abnormalities. As such, these end points supported the conclusion of the main regression analysis, that is, that there was no significant QT prolongation. Analysis of HR, PR interval, and QRS duration also revealed no adverse findings. As edaravone does not affect RR (HR), it is considered that correction with QTcF was adequate normalization.

There is no specific research available regarding thorough QT studies of edaravone. Therefore, for the first time, our trial conducted a proper evaluation of potential QT prolongation with edaravone. Importantly, and as described previously, such a postmarketing thorough QT study of edaravone was considered necessary to exclude any small QT prolongation effects (10-millisecond threshold), and it should be remembered that exposure-response analysis is particularly appropriate for use as the main analysis for assessing the QTc interval prolongation risk.

Previous PK studies in subjects with renal impairment revealed no clinically significant differences in edaravone AUC and  $C_{\max}$  (Mitsubishi Tanabe Pharma Corporation, data on file). Similar data were also observed in previous PK studies in subjects with hepatic impairment, although these studies have not yet been published (Mitsubishi Tanabe Pharma Corporation, data on file). Other high-clinical-exposure scenarios of edaravone were not detected.<sup>12</sup> Importantly, when using a suprathreshold dose of edaravone, as in our trial, edaravone can be evaluated at sufficiently high exposure levels (ie, at about 7 times higher than those in the therapeutic dose), which can cover the highest concentration anticipated during normal clinical use. Thus, a positive control was not necessary in our study because the suprathreshold test dose of edaravone was high enough, and reasonable assessments could be made according to ICH E14 and the scientific white paper guidance.<sup>13,14,18</sup> Moreover, our study had a sufficient number of electrocardiographic evaluation time points, with triplicate electrocardiographic data extraction at each time point with matched PK sampling, and was able to adequately evaluate the lack of associated hysteresis.

Our trial evaluated PK after administration of edaravone 60 mg over 60 minutes, which is a therapeutic dose, for the first time. However, in all cases, blood sampling for PK assessment deviated from the prescribed time of matching electrocardiographic measurement by  $\geq 5$  minutes, due to the prioritization of electrocardiographic measurement over PK sampling. As edaravone is eliminated rapidly from plasma, a “shift” of  $\geq 5$  minutes resulted in lower observed concentrations than the actual concentrations at specified time points, especially around  $C_{\max}$  at the end of edaravone infusion. To estimate actual  $C_{\max}$  and AUC, a 3-compartment model

analysis was employed. The observed geometric  $C_{\max}$ , which was used for assessment of  $\Delta\Delta\text{QTcF} < 10$  milliseconds and shown as  $C_{\max}$  in the noncompartmental analysis, was lower than the actual  $C_{\max}$  estimated by 3-compartment model analysis (Table 2). Nonetheless, we consider that any potential effect of edaravone on QT prolongation was properly assessed because exposure-response analysis was performed at lower-than-actual edaravone levels; subsequently, the slope for the relationship between  $\Delta\text{QTcF}$  and edaravone concentration was not underestimated. Overall, our direct evaluation with a therapeutic and suprathreshold dose of edaravone showed that there is no small QT prolongation effect ( $< 10$  milliseconds).

Goodness-of-fit plots, such as the one shown in Figure 2A, supported model validity for the regression relationship between  $\Delta\text{QTcF}$  and edaravone concentration, except for the quantiles of concentration and  $\Delta\text{QTcF}$  overlaid with the slope of the final model and individual data (Figure 2B), where the decile plots deviated from the straight line predicted by the model. The quantile plot of observed data overlaid with the model prediction suggested in the recent scientific white paper<sup>18</sup> may be useful to detect the misspecified model; however, model prediction will not fit the observed quantile plot if the average time effect is not 0. In our study, the estimated time effect was large, as suggested by the mean QTcF by time point (Figure 3A). The higher concentrations in Figure 2B correspond to the relatively higher  $\Delta\text{QTcF}$  of  $\leq 4$  hours in Figure 3A. Low concentrations correspond to the relatively lower  $\Delta\text{QTcF}$  of  $> 4$  hours in Figure 3A.

To obtain a visual fit for the quantile plot of observed data and the model prediction line with a large time effect, adjustment for the time effect is suggested.<sup>18</sup> Thus, a plot was created for each time point with adjustment for the time effect on each predicted line, in which the linear fit conformed to goodness-of-fit plots (Figure S1A–L). This was found to be an efficient approach to interpreting the quantile plot of observed data with the model prediction when the model contained large time effects. As this was a 3-way crossover study, a similar concentration-QTcF analysis could also be conducted with  $\Delta\Delta\text{QTcF}$  as the dependent variable, which enables exclusion of time effects from the linear mixed-effect model. The model analysis with  $\Delta\Delta\text{QTcF}$  provided quantiles of concentration and  $\Delta\Delta\text{QTcF}$  overlaid with slope of the final model (Figure S2), indicating no effect of edaravone on QTcF and supporting model validity and conclusions from the original model analysis. It is possible that the observed large time effect for QTcF was due to the time change in HR. Although the actual reasons are unclear, potential factors influencing the time change in HR could include taking food 4 hours after the start of edaravone infusion (after

an overnight fast), daytime activity levels (due to a reduced assessment schedule during daytime), or daily circadian rhythms.<sup>21,22</sup> Overall, it should be noted that because the edaravone and placebo groups showed similar HR and QTcF, it is clear that there was no drug effect and that the time effect for QTcF did not influence confirmation of the lack of drug effect on QTcF.

## Conclusion

These results indicate that edaravone, at concentrations up to about 7 times greater than those at a therapeutic dose, does not produce clinically meaningful QT prolongation as defined by ICH E14 guidance. Thus, edaravone has no clinically relevant effects on ECGs and, specifically, does not prolong cardiac repolarization.

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## Conflicts of Interest

H.S., S.I., M.E., Y.N., K.Y., T.N., M.K., M.A., and K.K. are employees of Mitsubishi Tanabe Pharma Corporation, Tokyo, Japan; there are no other conflicts of interest to disclose.

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## Author Contributions

T.N. and M.K. were involved in study design, conduct and data collection; M.E., Y.N., and S.I. were involved in study design, data analysis and interpretation, and writing or reviewing the manuscript; K.K. was involved in study design, data interpretation, and writing or reviewing the manuscript; K.Y. and M.A. were involved in study design, conduct and data collection, and writing or reviewing the manuscript; and H.S. was involved in data analysis and interpretation, writing or reviewing the manuscript, and final approval of the manuscript for submission.

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### Supplemental Information

Additional supplemental information can be found by clicking the Supplements link in the PDF toolbar or the Supplemental Information section at the end of web-based version of this article.