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Effects of differences in housing conditions on the evaluation of drug-induced QT prolongation in cynomolgus monkeys

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Objective

In recent years, there has been a growing emphasis on housing social animals, such as cynomolgus monkeys, in groups from the perspective of animal welfare. Furthermore, cardiovascular studies in safety pharmacology are now required to be carried out in group settings. Telemetry systems used in recent safety pharmacology studies allow for the simultaneous collection of cardiovascular data from individual animals that are housed in groups. In cardiovascular studies, prolongation of the QT interval is the most important evaluation parameter for assessing the risk of proarrhythmic effects. Since the QT interval is influenced by heart rate (RR interval) and exhibits diurnal variations, it can also be affected by changes in the housing environment, such as individual and group housing. In this study, we compared the effects of different housing conditions, specifically individual housing, pair housing, and group housing, on the evaluation of the QT interval.

Materials and Methods

[Animals]

4males cynomolgus monkeys (*Macaca fascicularis*) implanted with telemetry transmitters †

[Cages]

Individual housing

680W x 608D x 770Hmm

Pair housing

Two cages connected

Group housing

2340W x 1800D x 2050Hmm

[Frequency and Dosing Schedule]

▽ : Dosing
○ : Acclimation to the housing environment
1week

[Test Article]

Moxifloxacin (Avelox®,Bayer Yakuhin, Ltd.)

[Group Composition]

Treatment	Dose level (mg/kg)	Dosing volume (mL/kg)	Conc. (mg/mL)	Number of animals
Vehicle*1	0	5	0	4 or 3
Moxifloxacin	25	5	5	
	50	5	10	
	100	5	20	

*1: 0.5%MC

[Dosing Method]

Three doses/concentrations of Moxifloxacin were (25, 50, and 100 mg/kg) + vehicle was evaluated in the following order: individual housing, and pair housing, using a Latin square design. After the evaluation of pair housing, group housing was evaluated. In the group housing, one animal was excluded because it incurred a bite injury during the acclimation period to the housing environment, and three animals were used to evaluate the three doses (25, 50, and 100 mg/kg) of the positive control substance + vehicle using a crossover method.

[Data Analysis]

Data were collected using a telemetry system (PhysioTel® Digital /Ponemah [Harvard Bioscience, Inc]) from 1 hour before dosing until 24 hours after dosing. QTc intervals were calculated using an individual correction formula (QTci) [1]for 15 minutes before and after dosing (30 minutes in total) at 1, 2, 4, 6, 8, and 24 hours after dosing.

[Toxicokinetics]

(1)Blood concentrations of moxifloxacin were measured.
Time points of blood sampling
1 , 2, 4, 6, 8, and 24 hours after dosing

(2)Blood sampling method
Blood sampling volume: About 0.5 mL/point
Blood sampling site: Cephalic vein
Anticoagulant: Heparin sodium
Blood samples collected completion of each evaluation time point using disposable syringe treated with heparin sodium, immediately put into the sampling tubes, and cooled on ice.

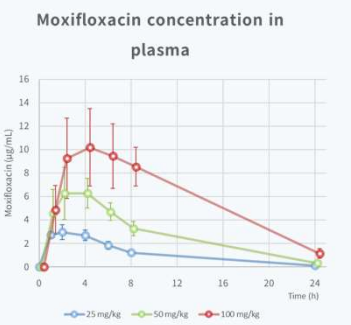
Approximately one month before the telemetry study, blood drug concentrations were measured at the same time point as the telemetry evaluation points.

(3) Blood samples was immediately centrifuged, and plasma sample was obtained from each animal.
Centrifugation: 10,000 × g for 3 minutes at 4°C

†: This study was conducted under the approval of the institutional official according to the following guideline of the test facility: Guidelines for Animal Studies.

Results

[Toxicokinetics]



- As a result of blood concentration measurement, T_{max} was 2.3 hours for the 25 mg/kg group, 3.5 hours for the 50 mg/kg group, and 4.5 hours for the 100 mg/kg group.

Moxifloxacin concentration in plasma

25 mg/kg	Plasma concentration (μg/mL)							T_{max}	C_{max}
Animal No.	pre	1 h	2 h	4 h	6 h	8 h	24 h	(h)	(μg/mL)
M001	<0.05	2.64	2.70	2.19	1.43	0.996	0.108	2.0	2.70
M002	<0.05	3.01	2.37	2.62	2.11	1.37	0.131	1.0	3.01
M003	<0.05	2.86	3.87	2.73	1.76	1.25	0.142	2.0	3.87
M004	<0.05	2.42	2.97	3.25	2.12	1.30	0.107	4.0	3.25
Mean	0	2.73	2.98	2.70	1.86	1.23	0.122	2.3	3.21
SD	-	0.26	0.64	0.44	0.33	0.16	0.017	1.3	0.50

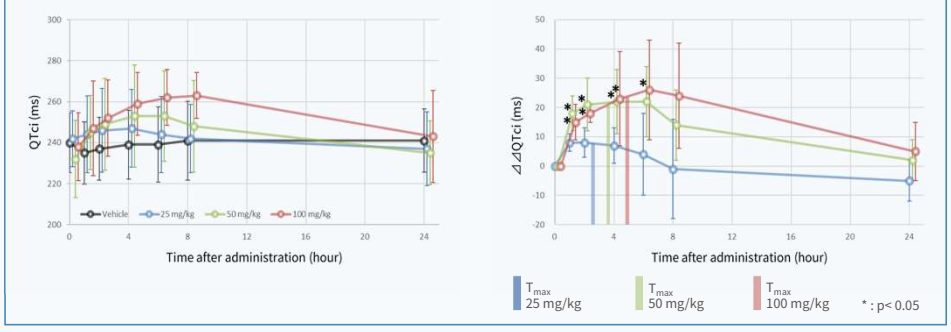
50 mg/kg	Plasma concentration (μg/mL)							T_{max}	C_{max}
Animal No.	pre	1 h	2 h	4 h	6 h	8 h	24 h	(h)	(μg/mL)
M001	<0.05	1.65	3.66	4.43	3.87	2.77	0.275	4.0	4.43
M002	<0.05	5.86	5.99	6.96	4.58	2.85	0.335	4.0	6.96
M003	<0.05	6.00	9.08	6.75	4.74	3.40	0.368	2.0	9.08
M004	<0.05	4.85	6.39	7.02	5.69	4.11	0.375	4.0	7.02
Mean	0	4.59	6.28	6.29	4.72	3.28	0.338	3.5	6.87
SD	-	2.03	2.22	1.25	0.75	0.62	0.046	1.0	1.90

100 mg/kg	Plasma concentration (μg/mL)							T_{max}	C_{max}
Animal No.	pre	1 h	2 h	4 h	6 h	8 h	24 h	(h)	(μg/mL)
M001	<0.05	2.50	4.56	5.49	5.34	6.52	1.67	8.0	6.52
M002	<0.05	3.74	9.08	13.0	10.5	8.94	0.802	4.0	13.0
M003	<0.05	6.71	12.5	10.4	11.0	8.20	0.891	2.0	12.5
M004	<0.05	6.51	10.9	11.9	11.0	10.5	1.25	4.0	11.9
Mean	0	4.87	9.26	10.2	9.46	8.54	1.15	4.5	11.0
SD	-	2.08	3.43	3.3	2.76	1.65	0.40	2.5	3.0

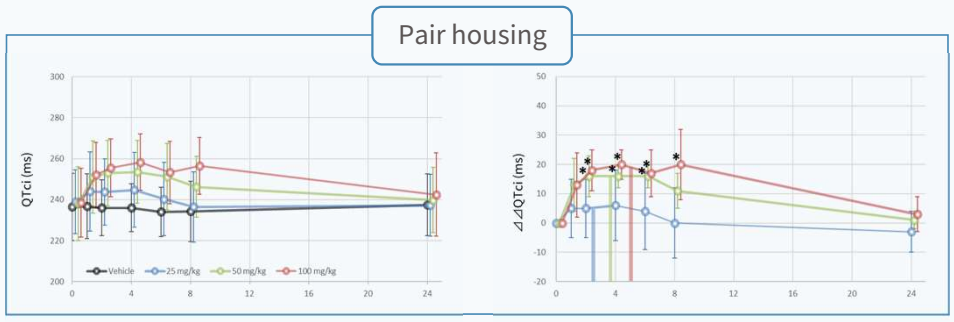
25 mg/kg 50 mg/kg 100 mg/kg C_{max}

[QT prolongation]

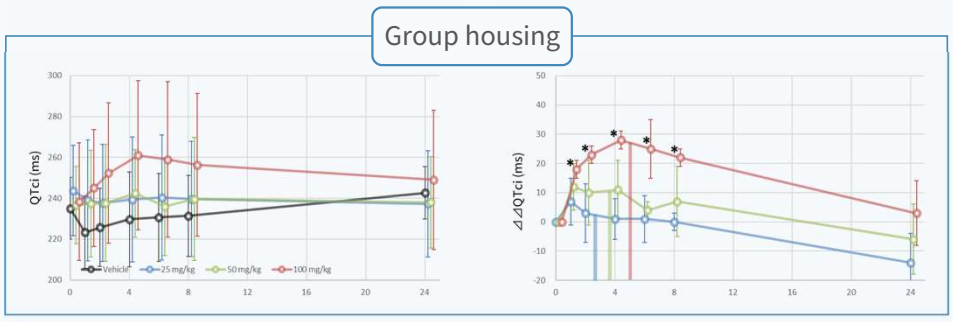
Individual housing



Pair housing



Group housing



- In multiple comparison tests (Dunnett's test), the 50 mg/kg and 100 mg/kg groups showed significant increases in both individually-housed and pair-housed conditions.
- In the group-housed condition, significant differences were observed in the 100 mg/kg group, but not in the 50 mg/kg group.
- No significant difference was observed in the 25 mg/kg group in either housing environment.
- In the 50 mg/kg group in the group-housed condition, the peak of QT prolongation exceeded the standard deviation and was observed earlier than the T_{max} of Moxifloxacin.

[RR interval]

- Statistical analysis of SD values of RR intervals showed no significant difference between the individually- and pair-housed conditions.
- When comparing individually-housed and group-housed conditions, the group-housed condition showed significantly higher values in the vehicle and 25 mg/kg groups.
- When comparing pair-housed and group-housed conditions, the group-housed condition showed significantly higher values in the vehicle, 25 mg/kg, and 50 mg/kg groups.
- No significant difference was observed in the 100 mg/kg group.

[C-QTc analysis]

- In the C-QTc analysis, the regression lines for individually-, pair-, and group-housed conditions showed positive slopes and were close to each other. The P values were consistent throughout all conditions.

Conclusion

The RR variation was larger in the group-housed condition compared to the other two conditions, which is thought to be the reason why no significant difference was observed in the QT prolongation effect in the group-housed 50 mg/kg group. As a result of the C-QTc analysis, the slopes of the regression lines were similar: 2.2 for the individually-housed condition, 1.9 for the pair-housed condition, and 2.3 for the group-housed condition. These results suggest that, although the heart rate (RR interval) varies depending on the housing conditions, the QT prolongation can be accurately evaluated using the C-QTc analysis.

[Reference]

[1] Holzgrefe HH, Cavero I, Gleason CR, Warner WA, Buchanan LV. Novel probabilistic method for precisely correcting the QT interval for heart rate in telemetered dogs and cynomolgus monkeys. J. Pharmacol. Toxicol. Methods. 2007; 55, 159-75.