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Investigation of effects of the known QT-prolonging drugs on the central nervous, cardiovascular, and respiratory systems in beagle dogs using the simultaneous evaluation method for the safety pharmacology core battery

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Introduction

Objectives of the safety pharmacology core battery are to investigate adverse side effects of new chemical entities on vital functions, that is, the central nervous system (CNS), cardiovascular system (CV), and respiratory system (RES). Different individuals and sometimes even different animal species are currently used for each target function; this fact could lead to more animal sacrifice, time, and cost spent in a study and elimination of possibility to detect inter-functional effects of a compound. It would be advantageous if effects of a compound on the said three functions are simultaneously tested in identical individuals.

We recently developed an innovative system which allows simultaneous evaluation of a compound's effects on CNS, CV and RES in identical free-moving dogs. First, we housed dogs in the whole-body-plethysmography chamber, an equipment of the simultaneous evaluation system, for up to 14 days, and found that the animals' condition were maintained healthily (Miyamori, et al., 2006, at the 33rd Annual Meeting of the Japanese Society of Toxicology). Subsequently, effects of morphine on CNS and RES were assessed using the system, and its measurement capability, including the higher resolution for respiratory parameters compared to that of the blood-gas analysis, was confirmed (Miyamori, et al., 2007, at the 80th Annual Meeting of the Japanese Physiological Society).

In this study, to further validate the system in terms of the cardiovascular function, we investigated effects of compounds, *dl*-sotalol and E-4031, that are known to prolong QT interval.

Methods

●*Animals*

Three male beagle dogs (21 months old, 11-12 kg, animals' code: 101-103) were used. Under anesthetization with an intravenous dose of thiopental sodium (25 mg/kg), the transmitter (TL11M2-D70-PCT; Data science international) of a telemetry system was fixed in the abdominal cavity; a catheter for blood-pressure measurement was placed in the abdominal aorta through the femoral artery; conductive wires were fixed to record AB lead electrocardiogram. An indwelling tube for arterial-blood collection was placed in the abdominal aorta through the femoral artery with an access port fixed on the back. An indwelling tube for intravenous infusion was placed in the caudal vena cava through the femoral vein with an access port fixed on the back.

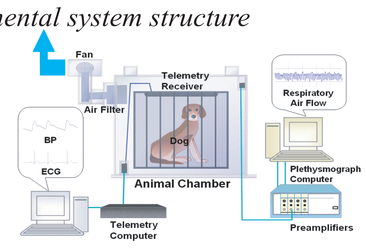
●*Administration*

dl-Sotalol at the doses of 2.5 and 10 mg/kg/hr and E-4031 at the dose of 1 mg/kg/hr were administered continuously for 4 hours using an infusion pump (Pegasus LIGHT, PEGASUS GMBH). Saline was administered at the rate of 1.25 mL/kg/hr in the same manner as a control.

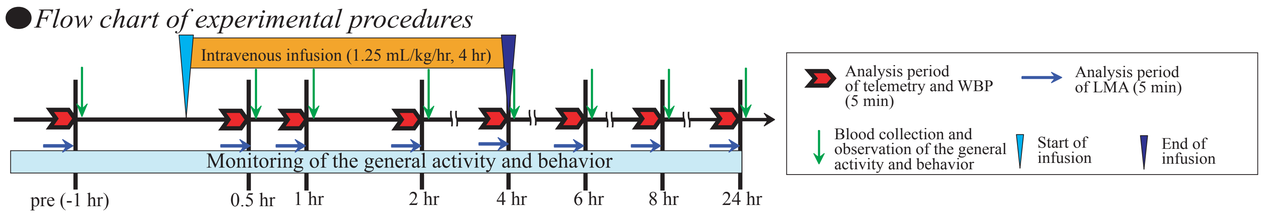
●*Recording and measuring systems*

Video recording system:
HDD-DVD recorder (DMR-EH55, Panasonic Co., Ltd.),
Charge-coupled device camera
Locomotor activity (LMA) measuring system:
SUPERMEX (Muromachi Kikai Co., Ltd.)
Telemetry system:
Open A.R.T 2.2 (Data science international),
HEM ver. 3.5 (NOTOCORD systems)
Whole body plethysmography (WBP) system:
Biosystem XA, ver.2.9.0 (Buxco Inc.)
Blood-gas analyzer:
ABL77 (Radiometer A/S)

●*Experimental system structure*



●*Flow chart of experimental procedures*



●*Experimental items*

【CNS】

Observation of the general activity and behavior

Posture, Behavior, Convulsion, Muscle tone, Righting reflex, Pupillary reflex, Palpebral reflex, Auditory response, Touch response, Pain response, Eyes, Nose, Fur, Mucosa, Salivation, Others

Monitoring of the general activity and behavior using charge-coupled device cameras

Posture, Behavior, Convulsion, Others

LMA measurement using SUPERMEX

Body temperature measurement using telemetry system

【RES】

WBP-data collection

Frequency, Tidal volume (V_T), Minute volume (V_M), Enhanced pause (Penh)

Measurement of blood gas

pH, Oxygen partial pressure (PaO_2), Carbon dioxide partial pressure ($PaCO_2$), Hemoglobin oxygen saturation (SaO_2)

【CV】

Telemetry-data collection

Mean blood pressure (MBP), Heart rate (HR), PR interval, QRS duration, QTc interval (Fridericia's formula: $QTc = QT/RR^{1/3}$)

●*Statistical analyses*

The repeated 2-way ANOVA was performed between the control group and each test-compound group in terms of measured values of the respective time points. Dunnett's test was performed, when a statistical difference between the groups was significant. The significance level was defined at 5% (2-tailed) for all cases. As for the result of the general activity and behavior, statistical analysis was not performed.

Results & Conclusion

【CNS】

Table 1. Effect of 4-hour infused *dl*-sotalol and E-4031 on general activity and behavior in dogs.

		n	pre (-2~0 hr)	Time after starting infusion (hr) ~0.5	~1	~2	~4	~6	~8	~24
Saline		3	NA	NA	NA	NA	NA	NA	NA	NA
dl-Sotalol	2.5 mg/kg/hr	3	NA	Vomiting (No. 101)	NA	NA	NA	NA	NA	NA
dl-Sotalol	10 mg/kg/hr	3	NA	NA	NA	NA	NA	NA	NA	NA
E-4031	1 mg/kg/hr	3	NA	Loose stool (No. 101)	Loose stool (No. 101, 103)	NA	Loose stool (No. 102)	NA	NA	NA

NA: Nothing abnormal detected
3-Digit number in parentheses represents the code of animals showed a sign.

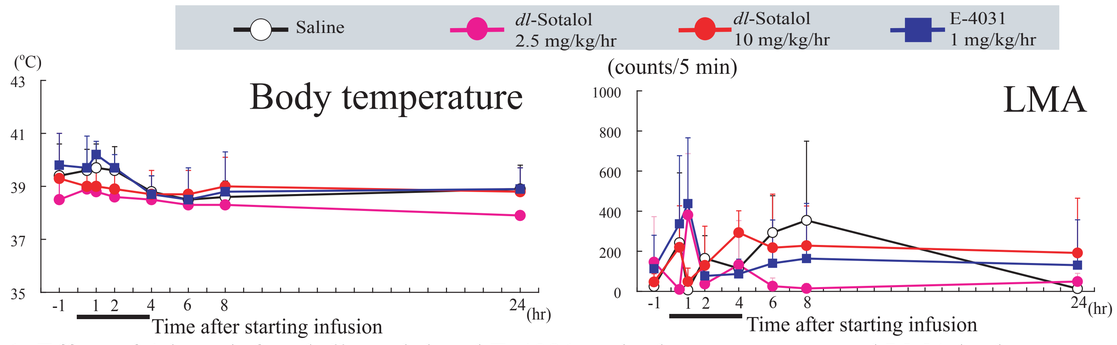


Fig. 1. Effect of 4-hour infused *dl*-sotalol and E-4031 on body temperature and LMA in dogs.
Each value represents the mean ± S.D. of 3 dogs. Horizontal bar represents the infusion period.
There was no significant difference from control.

【RES】

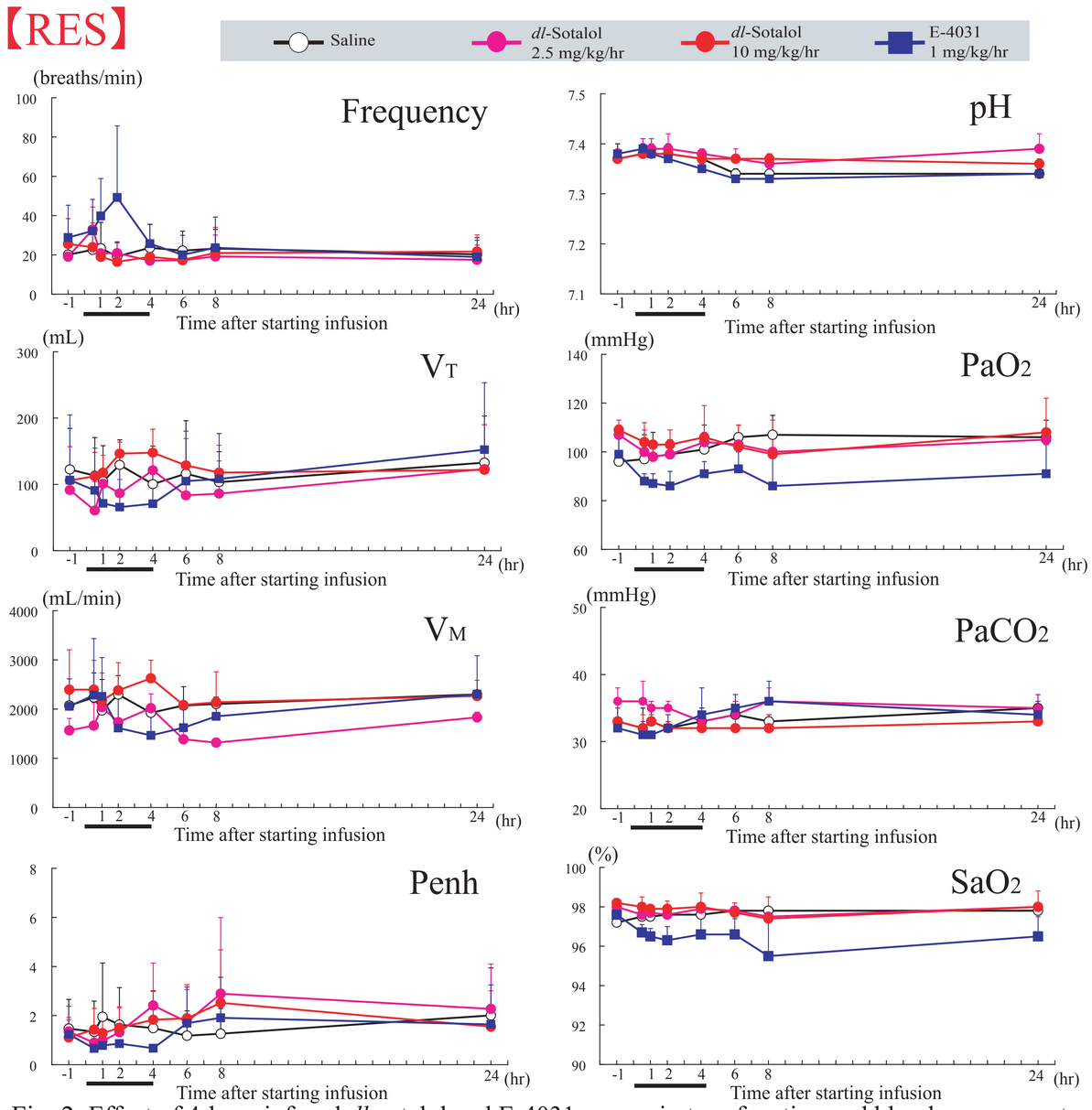


Fig. 2. Effect of 4-hour infused *dl*-sotalol and E-4031 on respiratory function and blood gas parameters in dogs.
Each value represents the mean ± S.D. of 3 dogs. Horizontal bar represents the infusion period.
There was no significant difference from control.

【CV】

Table 2. Effect of 4-hour infused *dl*-sotalol and E-4031 on heart rate, mean blood pressure and electrocardiogram in dogs.

		pre (-1 hr)	Time after starting infusion (hr) 0.5	1	2	4	6	8	24
HR (beats/min)									
Saline		80 ± 12	81 ± 8	81 ± 10	86 ± 12	83 ± 10	79 ± 9	81 ± 5	81 ± 11
dl-Sotalol	2.5 mg/kg/hr	83 ± 16	75 ± 8	77 ± 14	70 ± 8	74 ± 9	72 ± 11	71 ± 15	72 ± 6
dl-Sotalol	10 mg/kg/hr	91 ± 30	84 ± 16	86 ± 13	80 ± 14	81 ± 17	78 ± 12	82 ± 16	81 ± 19
E-4031	1 mg/kg/hr	102 ± 29	105 ± 16	112 ± 33	99 ± 21	98 ± 23	89 ± 16	91 ± 19	91 ± 21
MBP (mmHg)									
Saline		98 ± 7	104 ± 12	101 ± 8	106 ± 6	102 ± 11	99 ± 13	103 ± 9	91 ± 5
dl-Sotalol	2.5 mg/kg/hr	104 ± 9	98 ± 8	108 ± 18	96 ± 6	102 ± 17	95 ± 4	91 ± 9	95 ± 10
dl-Sotalol	10 mg/kg/hr	98 ± 7	103 ± 14	97 ± 10	96 ± 12	97 ± 11	92 ± 15	95 ± 13	93 ± 10
E-4031	1 mg/kg/hr	109 ± 23	114 ± 28	110 ± 29	98 ± 23	99 ± 22	95 ± 29	101 ± 27	98 ± 29
PR Interval (msec)									
Saline		130 ± 9	131 ± 15	130 ± 13	125 ± 12	130 ± 11	134 ± 10	130 ± 12	136 ± 14
dl-Sotalol	2.5 mg/kg/hr	131 ± 10	141 ± 12	137 ± 13	146 ± 12	145 ± 8	143 ± 12	141 ± 12	141 ± 13
dl-Sotalol	10 mg/kg/hr	139 ± 13	143 ± 9	148 ± 15	153 ± 16	151 ± 12	151 ± 13	149 ± 10	148 ± 9
E-4031	1 mg/kg/hr	119 ± 13	123 ± 6	116 ± 14	123 ± 7	137 ± 11	135 ± 15	137 ± 19	135 ± 13
QRS Duration (msec)									
Saline		39 ± 3	40 ± 3	37 ± 0	37 ± 1	38 ± 1	39 ± 1	39 ± 2	38 ± 2
dl-Sotalol	2.5 mg/kg/hr	39 ± 3	37 ± 1	38 ± 3	38 ± 2	38 ± 3	39 ± 2	38 ± 1	39 ± 2
dl-Sotalol	10 mg/kg/hr	39 ± 2	40 ± 3	41 ± 5	40 ± 4	40 ± 3	38 ± 2	40 ± 3	40 ± 3
E-4031	1 mg/kg/hr	39 ± 2	39 ± 2	39 ± 2	39 ± 3	40 ± 2	39 ± 2	39 ± 2	39 ± 3

Each value represents the mean ± S.D. of 3 dogs. There was no significant difference from control.

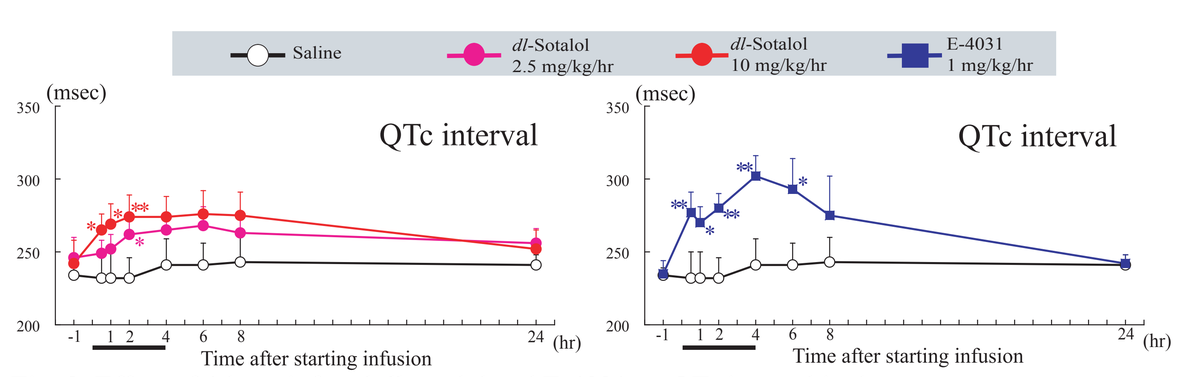


Fig. 3. Effect of 4-hour infused *dl*-sotalol and E-4031 on QTc interval in dogs.
Each value represents the mean ± S.D. of 3 dogs. Horizontal bar represents the infusion period. *: p<0.05, **: p<0.01; Statistically significant difference from control

<*dl*-Sotalol>

_dl_-Sotalol showed a dose-dependent prolongation of QTc interval; the effect was statistically significant 2 hours after starting 2.5-mg/kg/hr infusion and 0.5 to 2 hours after starting 10-mg/kg/hr infusion. As for the general activity and behavior, one of three animals had vomiting at the dose of 2.5 mg/kg/hr. Other than that, no parameters investigated in this study were affected by *dl*-sotalol.

<E-4031>

E-4031 significantly prolonged QTc interval from 0.5 to 6 hours after starting infusion. As for the general activity and behavior, loose stool was observed in all the animals. Other than that, no significant difference between the control and E-4031 groups was observed in the parameters investigated in this study.

In conclusion, the system we developed is feasible for evaluating effects of compounds on not only CNS and RES but also CV in the safety pharmacology core battery.