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Negative lusitropic property of nifekalant identified using ventricular pressure-volume loop analyses in anesthetized monkeys

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Abstract: The present study was conducted to clarify multiple cardiohemodynamic and electrophysiological properties including inotropic/lusitropic effects of nifekalant, a class III antiarrhythmic drug, in an isoflurane-anesthetized monkey. Nifekalant was administered intravenously at the therapeutic dose of 0.3 mg/kg over 10 min to male cynomolgus monkeys (n=4), followed by higher dose of 1 (n=3) or 3 mg/kg (n=1) that was limited due to arrythmogenicity. Left ventricular (LV) pressure-volume (PV) analysis revealed that the 0.3 mg/kg dose of nifekalant induced a negative lusitropic effect, recognized as a decrease in maximal rate of reduction in LV pressure and a prolonged isovolumic relaxation time. Nifekalant also decreased heart rate and increased LV end-diastolic pressure, but had no effects on the other cardiohemodynamic parameters examined. Electrophysiological analysis showed nifekalant at 0.3 mg/kg prolonged QT/QTc intervals with no evidence of arrhythmia. Higher doses of nifekalant induced ventricular arrhythmia in 3 out of 4 animals, in which both the short-term and long-term variability of the QT interval increased just before the occurrence of arrhythmia. In conclusion, a therapeutic dose of nifekalant had no effect on inotropic activity or cardiac compliance, whereas it showed negative lusitropic properties and QT/QTc prolongation in isoflurane-anesthetized monkeys. In addition, higher doses of nifekalant showed remarkable QT/QTc prolongation leading to arrhythmogenicity, which showed good accordance with clinical findings. Caution should be paid to negative lusitropic properties as well as arrhythmogenisity for the safe use of nifekalant.
Key words: load-independent, monkeys, negative lusitropy, nifekalant, pressure-volume loop

Introduction

Nifekalant is a pure potassium channel blocker, which mainly inhibits a rapid component of delayed rectifier potassium current (I_{Kr}), with clinically antiarrhythmic effects against ventricular arrhythmias [18, 31]. This drug prolongs the ventricular repolarization period and is categorized as a pure class III antiarrhythmic agent [25]. There has been substantial *in vivo* research on the electrophysiological properties of nifekalant attributable to its mechanisms of action. Specifically, its effects on the ventricular repolarization phase have been well-investigated. Anti-arrhythmic effects of nifekalant were demonstrated in the canine cardiopulmonary arrest model where it decreased transmural dispersion of repolarization in the left ventricle [32]. In the anesthetized open-chest atrioventricular (AV) block canine model, nifekalant had inferior proarrhythmic properties in com-

parison with *dl*-sotalol [25]. In contrast, the conscious chronic AV block canine model indicated that an oral administration of nifekalant at a 10-fold higher concentration than its clinically relevant antiarrhythmic dose induced remarkable prolongation of the QT interval leading to Torsades de Pointes (TdP) [24]. Clinical reports regarding electrophysiological disturbances in patients with heart failure treated with nifekalant because of drug-induced long QT syndrome (LQTS) have identified one of the most critical issues in nifekalant use [23]. Nifekalant possess a stronger potential to induce occurrence of ventricular tachycardia, including TdP, due to LQTS than other anti-arrhythmic drugs currently available [23]. Conversely, information on the hemodynamic properties of nifekalant has been limited to date. Nifekalant has been reported to have weaker effects on left ventricular (LV) pressure in an open-chest anesthetized canine model [25] and did not induce any significant changes in heart rate, mean blood pressure, cardiac output, or maximal upstroke velocity of LV pressure ($LVdP/dt_{max}$) in a canine myocardial infarction model [17]. In clinical use, it has been reported that there were little effects on the hemodynamics by nifekalant in patients with acute extensive infarction and severe ventricular dysfunction [27] or with ventricular tachyarrhythmia/fibrillation [21]. However, few data regarding the load-independent inotropic or lusitropic properties of nifekalant are available despite the possibility that potassium channel blocking by nifekalant induces changes in cardiac inotropy/lusitropy by altering Ca^{2+} kinetics in cardiomyocytes [9].

LV pressure-volume (PV) loop studies in the isoflurane-anesthetized monkey showed milrinone- and metoprolol-induced effects on cardiac inotropy and lusitropy, respectively [16]. Furthermore, *dl*-sotalol did not show any inotropic activity in the monkey PV loop analysis [16] because of its inhibitory effects on I_{Kr} and β-blocking activity [1, 2], suggesting the practical utility of the LV PV loop method to evaluate the cardiac inotropic or lusitropic potential of drug candidates at the pre-clinical stage.

In the present study, the effects of potassium channel inhibition on multiple cardiohemodynamic and electrophysiological properties including cardiac inotropy/lusitropy of nifekalant, a representative I_{Kr} blocker, was investigated using the LV PV-loop method in cynomolgus monkeys, which is an important animal used in non-clinical toxicological studies and in evaluation of phar-

macological safety [19, 30]. Since the significance of non-clinical assessment for drug-induced alterations in cardiovascular functions using non-human primates as well as dogs are increasing, especially in drugs possibly targeting for susceptible patients [5, 11], we selected cynomolgus monkeys because of available background data using PV loop method for representative inotropic/lusitropic drugs in this species [15, 16].

Materials and Methods

Drugs

Intravenous formulation of nifekalant (Shinbit® inj. 50 mg, TOA EIYO LTD., Fukushima, Japan) was diluted in 0.9% physiological saline (Otsuka Normal Saline, Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan). The dose formulation was prepared at the appropriate concentrations at a volume of 1.0 ml/kg before usage.

Animals

Cynomolgus monkeys (*Macaca fascicularis*) were obtained from the Oriental Yeast Co., Ltd. (Tokyo, Japan). A total of 4 male monkeys weighing approximately 4.3–5.0 kg at ages 4–5 years were used in this study. The animals were housed individually in a stainless-steel cage (width 594 mm × depth 870 mm × height 1,015 mm) until the start of the experiment under the following environmental conditions: room temperature, 24°C; relative humidity, 60%; illumination, 150–300 luces; lighting, 12-hour light (7:00 to 19:00) and ventilation, 10–15 air changes/h. The animals were fed 100 g commercial pellet for monkeys (PS-A; Oriental Yeast Co., Ltd., Tokyo, Japan) once a day in the morning after observation of their clinical signs. This study was conducted in compliance with the "Law Concerning the Protection and Control of Animals" (Japanese Law No. 105, October 1, 1973) and "Fundamental Guidelines for Proper Conduct of Animal Experiment and Related Activities in Organizations under the jurisdiction of the Ministry of Health Labour and Welfare" (Notification No. 0601001, issued by the Japanese Ministry of Health Labour and Welfare, dated June 1, 2006). The present study was carried out in accordance with the Research Standard Operating Procedures for animal experiments approved by the Ethics Review Committee for Animal Experimentation of Daiichi Sankyo Co., Ltd. (Tokyo, Japan) in compliance with regulations.

Induction and maintenance of anesthesia and surgical preparation

Monkeys were initially anesthetized with intramuscular administration of ketamine hydrochloride (Ketalar® Intramuscular 500 mg, Daiichi Sankyo Co., Ltd.) at 10 mg/kg and intubated with a cuffed endotracheal tube. Subsequently, 1 to 3% isoflurane (Escain®, Pfizer Japan Inc., Tokyo, Japan) vaporized with 100% oxygen was inhaled with a volume-cycled ventilator (anesthetic ventilator PRO-55S combined with PRO-55V, Acoma Medical Industry Co., Ltd., Tokyo, Japan), and the respiratory rate and tidal volume were set at 12 to 20 breaths/min and 12.5 to 30.0 ml/kg, respectively. Body temperature, oxyhemoglobin saturation measured by pulse oximetry, and end-tidal carbon dioxide were continuously monitored using a multi-functional physiological monitoring system (BioScope AM130, Fukuda M-E Kogyo Co., Ltd., Tokyo, Japan) and were sustained within the physiological range throughout the experiment by warming the animals with a forced-air warming system (3M™ Bair Hugger™ Warming Unit Model 750, 3M Co., MN, USA). After proper and stable anesthesia was established, fentanyl (Fentanyl Injection 0.1 mg "Daiichi Sankyo", Daiichi Sankyo Co., Ltd.) was infused to the animals at 0.01 to 0.1 ml/kg/h until the end of the experiment. In addition, the animals were paralyzed with intravenous administration of 0.1 to 0.3 ml of rocuronium bromide (ESLAX® Intravenous 25 mg/2.5 ml, MSD K.K., Tokyo, Japan) to arrest spontaneous respiration in order to create stable PV loops during occlusion. A heparinized catheter (Hemostasis Introducer, Nihon Kohden Corporation, Tokyo, Japan) was inserted through the right femoral artery for continuous monitoring of arterial blood pressure. A PV catheter (FTH-5018B-E248B, Transonic Scisense Inc., Ontario, Canada) was positioned in the left ventricle via the carotid artery to monitor LV pressure and volume. A venous occlusion catheter (NOK-3F080-W, Nipro Corporation, Tokyo, Japan) was positioned in the caudal vena cava via a catheter inserted in the left femoral vein.

Data acquisition and analyses

Pressure and volume of the left ventricle were monitored through a data acquisition system (ADV500 Admittance Pressure Volume Control Unit, Transonic Scisense Inc.). The lead II electrocardiogram (ECG) obtained from limb electrodes was monitored via a multi-functional ECG monitoring system (Cardisuny D700, Fukuda M-E Kogyo Co., Ltd.), and arterial blood pressure was obtained using a polygraph system (RM-6000, Nihon Kohden Corporation). The electrical-mechanical window (EMw), an index of torsadogenicity, was measured as the time difference between the end of LV contraction (electrical) and the end of the QT interval (mechanical). The $LVdP/dt_{max}$, maximal rate of the fall of LV pressure ($LVdP/dt_{min}$), LV end-systolic pressure (LVESP), and LV end-diastolic pressure (LVEDP) were recorded via the PV catheter. LVEDP was measured at the end of the atrial "kick" before the rapid rise of LV pressure. Families of LV PV loops were generated via the PV catheter during an acutely decreased preload produced by occlusion of the caudal vena cava for approximately 5 seconds. All of these parameters were continuously recorded into a physiological data acquisition system (Ponemah Physiology Platform, Data Science International, MN, USA).

Each hemodynamic and electrophysiological parameter was represented as the mean of 10 consecutive heartbeats. PR interval, QRS width, and QT interval were measured using ECG waveform recognition software (Ponemah Physiology Platform, Data Science International). Corrected QT intervals (QTc) for heart rate were calculated using Bazett's formula (QTcB = QT / [60,000 / RR] $^{1/2}$) [4], Fridericia's formula (QTcF = QT / [60,000 / RR] $^{1/3}$) [10], and Van de Water's formula (QTcV = QT − 0.087 × RR − 1,000]) [28]. QA interval was measured as the duration from the beginning of the Q wave to the onset of the arterial blood pressure pulse. The isovolumic relaxation time, tau, was calculated by the segment of the pressure contour between aortic valve closure and mitral valve opening, which were detected from the aortic and LV pressure waveforms. Results obtained from the families of LV PV loops during occlusion, the slopes of preload-recruitable stroke work (PRSW), the end-systolic PV relationship (ESPVR), and diastolic compliance (β), determined as the exponential fit of the end-diastolic PV relationship (EDPVR), were used to investigate the mechanical properties of the heart [7]. The slope of the PRSW was drawn by liner regression of cardiac stroke work and end-diastolic volume during occlusion. All these analyses were conducted using a physiological data acquisition system (Ponemah Physiology Platform, Data Science International). A Poincaré plot comparing QT_n versus QT_{n+1} was generated using a stable ECG of 51 consecutive beats just before arrhythmia occurrence the ECG at approximately 5, 10,

15 and 20 min after the initiation of dosing. The calculation method used was the same as that used to analyze the proarrhythmic potential of drugs in the AV block dog model [26]: short-term variability (STV) (=$\sum|QT_{n+1} - QT_n| / [50 \times \sqrt{2}]$) was determined as the mean orthogonal distance from the diagonal to the points of the Poincaré plot and long-term variability (LTV) (=$\sum|QT_{n+1} + QT_n - 2QT_{mean}| / [50 \times \sqrt{2}]$) was determined as the mean distance to the mean of the parameter parallel to the diagonal of the Poincaré plot. The coefficient of variation (CV) of the QT interval (SD / mean × 100, %) was also calculated using these to parameters [26].

Experimental protocol

The experiments were conducted individually for 4 animals and the cardiovascular variables were assessed in the following order. The ECG, arterial blood pressure, and the pressure and volume of the left ventricle were recorded under non-occlusion and sinus rhythm conditions. Next, families of LV PV loops were obtained during brief occlusions of the vena cava conducted in duplicate or triplicate, allowing the electrophysiological or hemodynamic parameters to return to the pre-occlusion status between occlusions. After a pre-drug control assessment, a low dose of 0.3 mg/kg of nifekalant was administered intravenously over 10 min and the cardiovascular variables were assessed at 5, 10, 15, and 20 min after the start of dosing. Then, an additional higher dose of 1 mg/kg (3 of 4 animals) or 3 mg/kg (1 of 4 animals) was administered and the ECG was assessed within 20 min after the initiation of dosing. In the first experiment (Animal No. 1), the arrhythmia occurred in 3 min after the start of administration at 3 mg/kg and it occurred frequently during the scheduled data acquisition period.

Although arterial pulse pressure had been observed, it was impossible to obtain stable LV PV loops during this period. Thus the higher dose level was reduced from 3 to 1 mg/kg for the remaining 3 animals (Animal Nos. 2, 3, and 4). The selected doses were expected to achieve those of plasma therapeutic levels in humans [14].

Measurement of plasma drug concentrations

A volume of 0.6 ml of blood was drawn from the left femoral artery to measure plasma drug concentrations at 5, 10, and 20 min after the start of the low-dose infusion. Plasma was prepared by centrifugation at 1,500 × g for 30 min at 4°C. Plasma nifekalant concentration was determined by liquid chromatography-tandem mass spectrometry (Waters 2795 Separations Module, Nihon Waters K.K., Tokyo, Japan; MS/MS, API 4000, AB SCIEX, MA, USA).

Statistical analysis

Data are presented as the mean ± SEM. Significance of the effects of the test drug on cardiovascular parameters was evaluated by one-way repeated-measures analysis of variance (ANOVA) to compare post-dose values versus pre-drug control values for each parameter. All statistical analyses were performed using the SAS® System Release 9.2 (SAS Institute Japan Ltd., Tokyo, Japan).

Results

Plasma drug concentrations

Plasma nifekalant concentrations at each time point are summarized in Table 1. The peak plasma nifekalant concentration in monkeys treated with the low dose was 424 ± 50 ng/ml (= 959 nmol/l).

Table 1. Plasma concentration of nifekalant in the isoflurane-anesthetized monkeys treated with 10 min-infusion of nifekalant at 0.3 mg/kg

	Animal No.	Sex	0.3 mg/kg/10 min		
			Time after the start of dosing (min)		
			5	10	20 (10)[a]
Plasma concentration (ng/ml)	No. 1	Male	360	365	456
	No. 2	Male	319	196	40.5
	No. 3	Male	485	540	60.3
	No. 4	Male	531	519	33.7
	Mean ± SEM		424 ± 50	405 ± 80	148 ± 103

SEM: standard error of the mean. [a] The numbers in parentheses indicate time after the finish of administration (min).
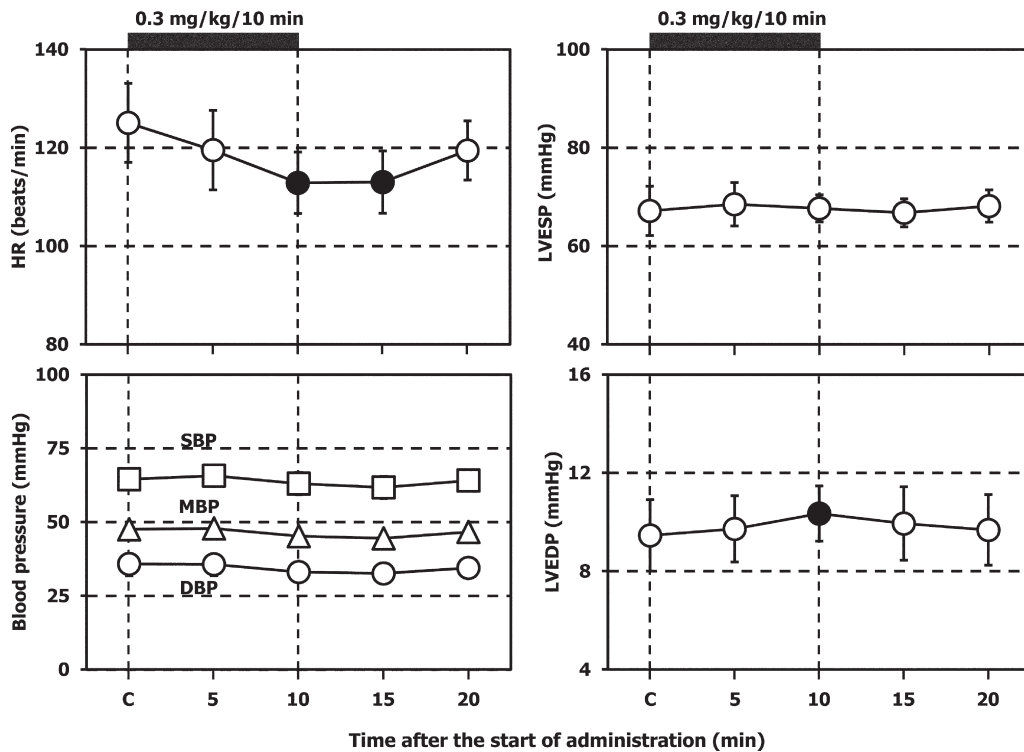
Fig. 1. Time-course of changes in heart rate (HR), systemic arterial blood pressure (systolic blood pressure [SBP, squares], diastolic blood pressure [DBP, circles], mean blood pressure [MBP, triangles]), LV end-systolic pressure [LVESP] and LV end-diastolic pressure [LVEDP] in cynomolgus monkeys treated with nifekalant. Data are presented as mean ± SEM (n=4). Closed symbols represent significant differences (*P*<0.05) from the pre-drug control (C) for each parameter.

Heart rate, systemic arterial pressure, and LV pressure

Time-course changes of heart rate, systemic arterial pressure, and LV pressure are summarized in Fig. 1. Pre-drug baseline values of heart rate and systolic, diastolic, and mean systemic arterial blood pressure, LVESP, and LVEDP were 125 ± 8 beats/min, 64 ± 2 mmHg, 36 ± 4 mmHg, 48 ± 4 mmHg, 67 ± 5 mmHg and 9.5 ± 1.5 mmHg, respectively. Nifekalant at 0.3 mg/kg decreased heart rate (113 ± 6 beats/min; a 10% reduction from baseline) and increased LVEDP (10.3 ± 1.1 mmHg; a 12% increase from baseline).

Mechanical properties of the left ventricle

Typical traces of the LV PV loops and the slopes of the ESPVR and EDPVR at baseline and at the end of administration of the low dose of nifekalant are shown in Fig. 2. There were no clear differences between these slopes before and after administration of nifekalant. The slopes of the portions of PV loops during the isovolumetric periods were vertical.

Load-independent inotropic parameters

Time-course changes of the slopes of PRSW and ESPVR are summarized in Fig. 3. Pre-drug baseline values of slopes of the PRSW and ESPVR were 39.5 ± 2.4 mmHg and 5.0 ± 1.1 mmHg/ml, respectively. No significant changes were detected in comparison with their corresponding pre-drug control values in either parameter.

Other inotropic parameters

Time-course changes of the LVdP/dt$_{max}$, QA interval, and contractility index are summarized in Fig. 4. The pre-drug baseline values of LVdP/dt$_{max}$, QA interval, and contractility index were 1,756 ± 519 mmHg/s, 133 ± 7 ms, and 62.7 ± 13.9 s^{-1}, respectively. No significant changes in any parameter were observed in comparison with their corresponding pre-drug control values.

Lusitropic parameters

Time-course changes of LVdP/dt$_{min}$ and tau are summarized in Fig. 5. The pre-drug baseline values of LVdP/
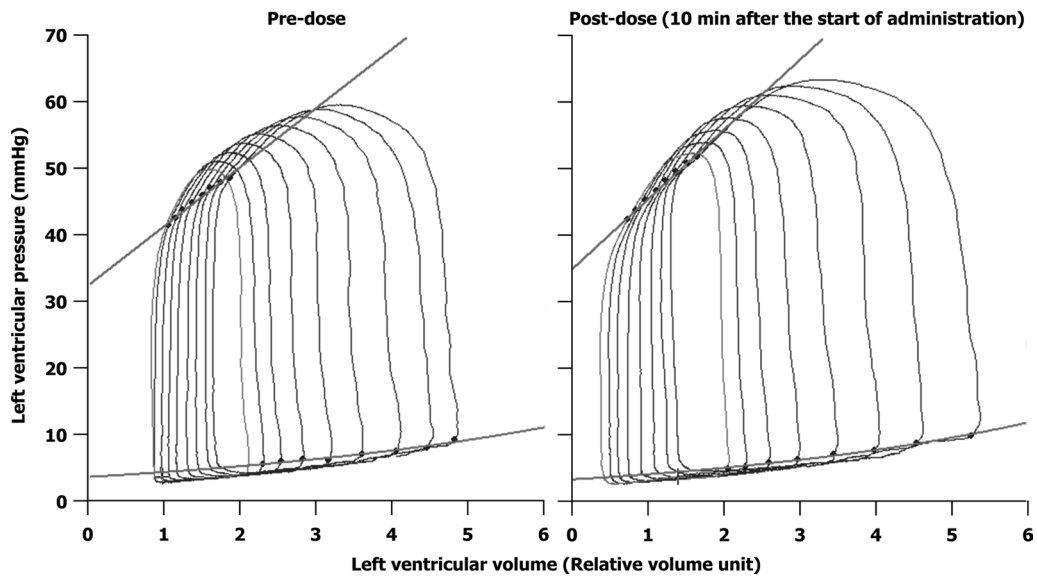
Fig. 2. Typical tracings of the pressure-volume loop before dosing (left) and after dosing (right) from a single cynomolgus monkey treated with a high dose of nifekalant. The straight lines intersecting the end-systolic points show the slope of the end-systolic pressure volume relationship. The downward convex curves intersecting the end-diastolic points show the slope of the end-diastolic pressure volume relationship.
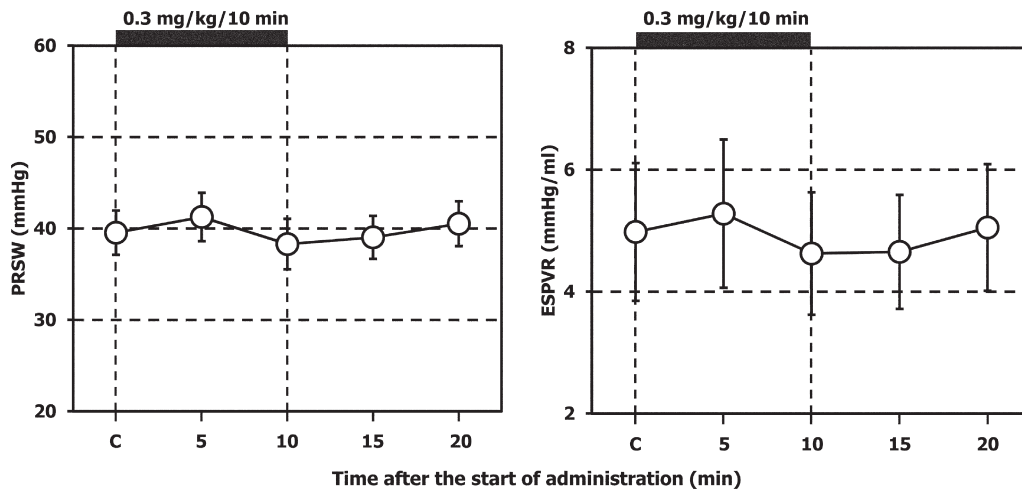

Fig. 3. Time-course of changes in preload-recruitable stroke work (PRSW) and the end-systolic pressure volume relationship (ESPVR) in cynomolgus monkeys treated with nifekalant. Data are presented as mean ± SEM (n=4). No significant differences were observed in post-drug values from the pre-drug control (C) for each parameter.

dt_{min} and tau were $-4,093 \pm 1,274$ mmHg/s and 33.2 ± 3.3 ms, respectively. Nifekalant decreased $LVdP/dt_{min}$ and increased tau ($-2,866 \pm 767$ mmHg/s and 43.3 mmHg/ml; a 25% reduction and 31% increase from baseline, respectively).

Stiffness parameters

Time-course changes of the EDPVR slope and EDPVR_β are shown in Fig. 6. Pre-drug baseline values of the EDPVR slope and EDPVR_β were 2.65 ± 1.12 mmHg/ml and 0.126 ± 0.043 mmHg/ml, respectively. No significant changes were observed in either parameter in comparison with their corresponding pre-drug control values.
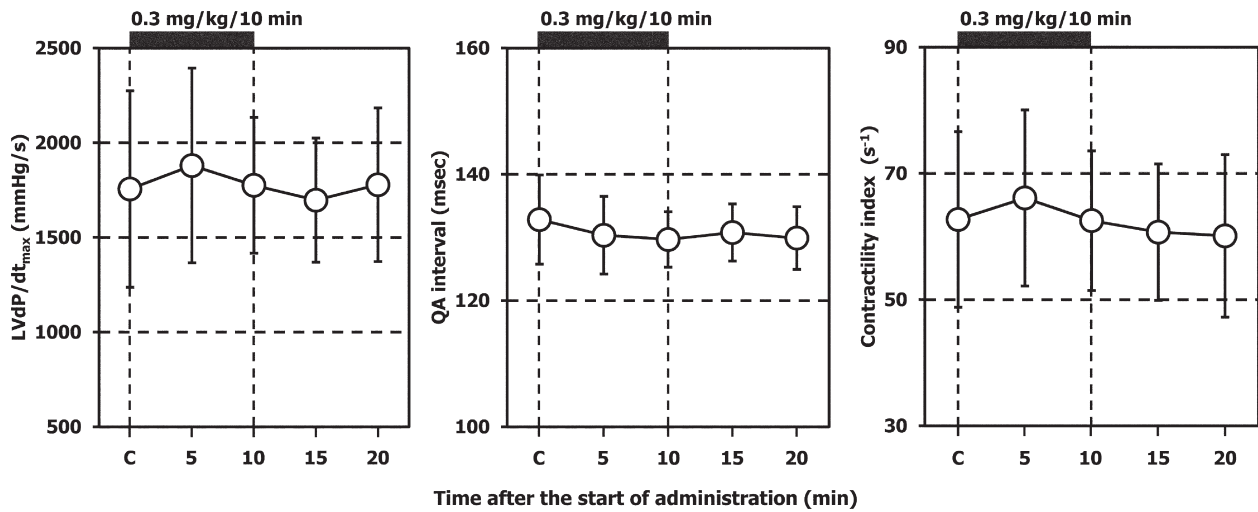
Fig. 4. Time-course of changes in the maximal upstroke velocity of the left ventricular pressure, QA interval, and contractility index in cynomolgus monkeys treated with nifekalant. $LVdP/dt_{max}$: maximum upstroke velocity of left ventricular pressure. Data are presented as mean $\pm$ SEM (n=4). No significant differences were observed in post-drug values from the pre-drug control (C) for each parameter.
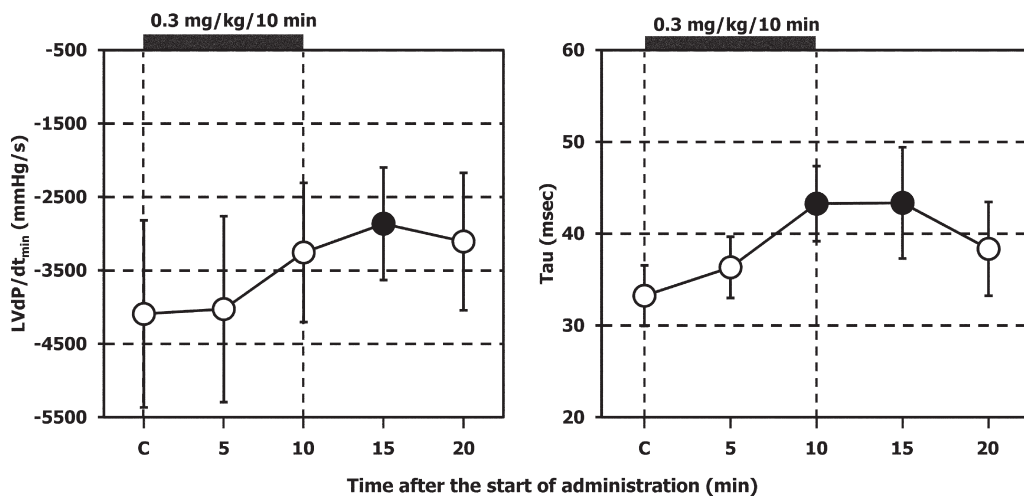

Fig. 5. Time-course of changes in the maximal rate of fall of left ventricular pressure ($LVdP/dt_{min}$) and time constant for isovolumic relaxation (tau) in cynomolgus monkeys treated with nifekalant. Data are presented as mean $\pm$ SEM (n=4). Closed symbols represent significant differences ($P<0.05$) from the pre-drug control (C) for each parameter.

ECG parameters

Time-course changes of the ECG parameters are shown in Fig. 7. Pre-drug baseline values of the PR interval, QRS width, QT interval, QTcB, QTcF, and QTcV were 86 $\pm$ 3, 33 $\pm$ 0, 287 $\pm$ 11, 413 $\pm$ 15, 365 $\pm$ 12, and 331 $\pm$ 10 ms, respectively. Nifekalant prolonged QT interval, QTcB, QTcF and QTcV (395 $\pm$ 26, 539 $\pm$ 24, 486 $\pm$ 25, and 436 $\pm$ 24 ms; with increases of 39%, 32%, 34%, and 32% from baseline, respectively).

Torsadogenic index

Time-course changes of the EMw are shown in Fig. 7. Pre-drug baseline value of EMw was 15 $\pm$ 21 ms. EMw tended to decrease after nifekalant administration, although no significant changes were observed in EMw (-58 ± 55 ms; a 764% reduction from baseline). Pre-drug baseline values of STV, LTV, and CV were 1.5 $\pm$ 0.9 ms, 1.9 $\pm$ 1.0 ms, and 0.8 $\pm$ 0.5%, respectively. Representative examples of Poincaré plots are shown in Fig. 8. Nifekalant at high doses induced premature ventricular
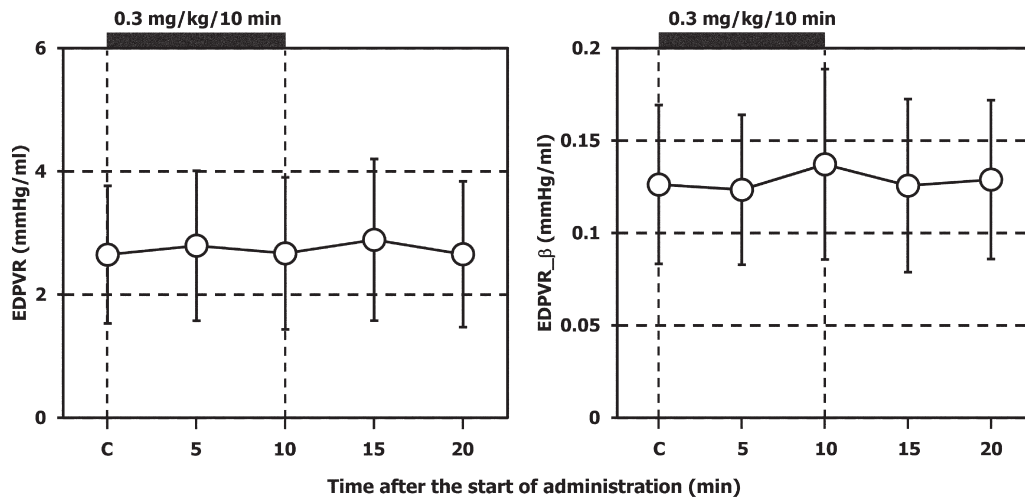
Fig. 6. Time-course of changes in the slope of the end-diastolic pressure volume relationship (EDPVR) and the stiffness constant (β) derived from EDPVR (EDPVR_β) in cynomolgus monkeys treated with nifekalant. Data are presented as mean ± SEM (n=4). Closed symbols represent significant differences ($P<0.05$) from the pre-drug control (C) for each parameter.
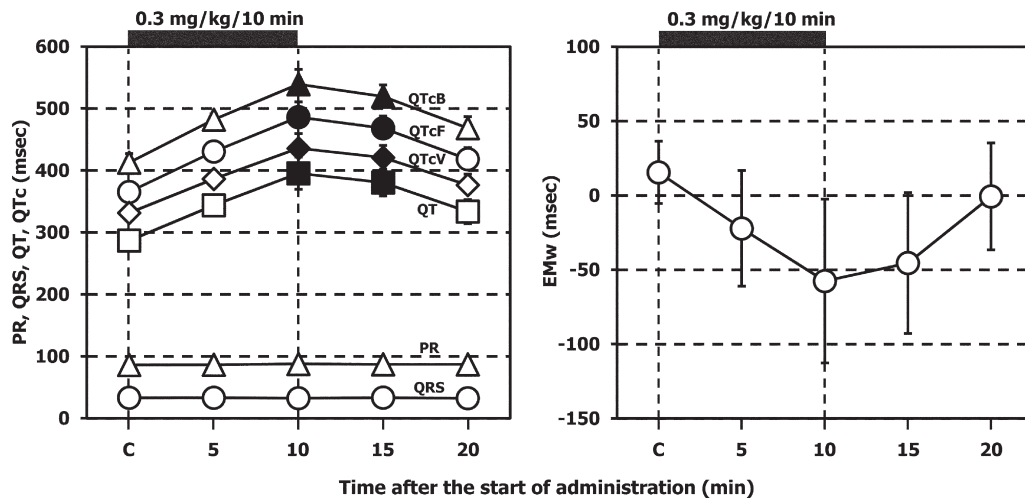

Fig. 7. Time-course of changes in the PR interval (triangles), QRS width (circles), QT interval (squares), and QT interval corrected by Bazett's formula (QTcB, triangles), Fridericia's formula (QTcF, circles) or Van de Water's formula (QTcV, diamonds), and electrical-mechanical window (EMw) in cynomolgus monkeys treated with nifekalant. Data are presented as mean ± SEM (n=4). Closed symbols represent significant differences ($P<0.05$) from the pre-drug control (C) for each parameter.

beats in 3 of 4 animals; in one animal (Animal No. 1), the arrhythmia occurred in 3 min after the start of administration and did not return to normal rhythm. In the two animals (Animal Nos. 2 and 3), the arrhythmia occurred in 5 or 6 min and lasted for around 13 min. STV, LTV, and CV increased in these 3 animals just before arrhythmia occurrence, whereas none of the other parameters showed any clear differences from the animal without arrhythmia throughout the experiment.

Discussion

In the present study, the inotropic and lusitropic profiles of nifekalant in isoflurane-anesthetized monkeys were clarified by the LV PV loop method, in addition to the evaluation of its hemodynamic and electrocardiographic properties. Isoflurane, which was used to maintain the animals under anesthetic conditions during the experiment, is known to exert negative inotropic activ-
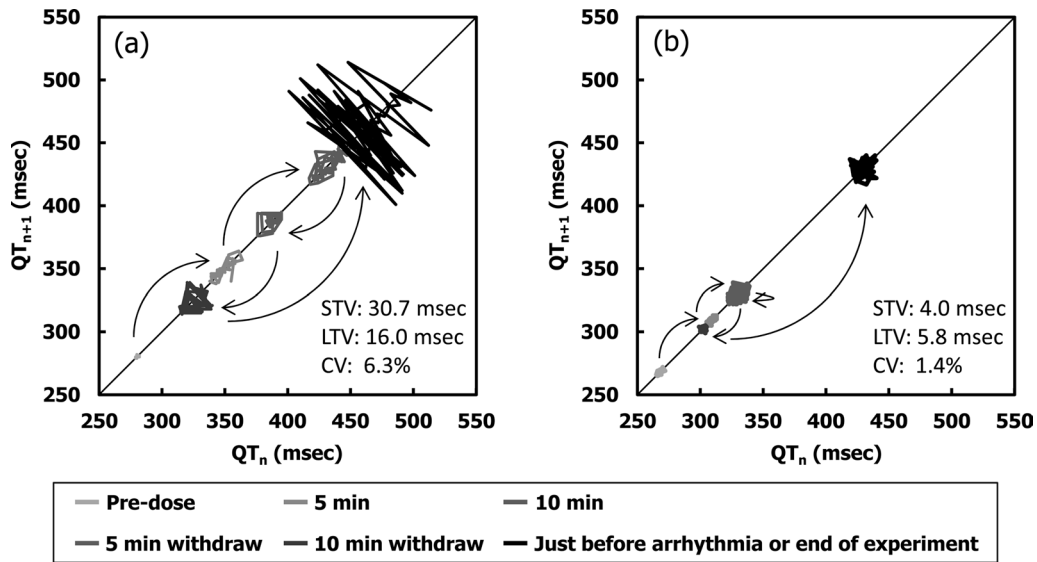
Fig. 8. The Poincaré plots of the QT interval measured after 51 beats. The plots show pre-dose, and 5 min and 10 min after dosing of nifekalant at low dose, 5 min and 10 min after withdrawal, and just before arrhythmia or end of experiment at a high-dose in (a) an animal with arrhythmia (animal No. 2) or (b) an animal without arrhythmia (animal No. 4). The short-term variability (STV), long-term variability (LTV), and coefficient of variation (CV) of the QT interval preceding arrhythmia (a) or at the end of the experiment (b) are presented in the box.

ity [3, 13]. Isoflurane is also known to reduce calcium and potassium channel currents in voltage-clamped vascular muscle cells derived from the canine coronary artery, and these functions might cause vasodilatory conditions or enhanced sensitivity to I_{Kr} blocking action by nifekalant [6]; however, the administration of the vehicle control, 0.9% physiological saline, did not induce any significant changes in this isoflurane-anesthetized monkey as previously reported [16]. To the best of our knowledge, ours is the first *in vivo* study using non-rodents where nifekalant was evaluated using load-independent inotropic parameters.

The therapeutic plasma concentrations of nifekalant in humans have been reported to be approximately 0.5 μg/ml [14, 20]. The plasma concentration at 0.3 mg/kg in the present study corresponded to the therapeutic level and therefore the dose selection was appropriate to adequately demonstrate the pharmacological effects of nifekalant.

In line with the effects on the slope of PRSW, the $LVdP/dt_{max}$, ESPVR, QA interval, and CI were not changed after administration of nifekalant. Nifekalant lengthened ventricular repolarization (e.g., prolonged QT interval and QTc, regardless of the correction formula), which could be explained by the blockade of

cardiac I_{Kr}. These findings suggested that nifekalant had no detectable inotropic activity at the therapeutic plasma concentrations tested in which QT/QTc prolongation was observed, as was the case with the concentrations used in the canine treated with nifekalant [12, 17, 25]. Potassium channel inhibition alone probably had no effects on cardiac inotropy, although it might have had a neutralizing potential against positive inotropy by indirectly modulating the intracellular Ca^{2+} concentration by maintaining intracellular K^+ concentration during cardiac repolarization [16]. Although the drug decreased the absolute value of the left ventricle relaxation velocity, $LVdP/dt_{min}$ and prolonged the time constant for isovolumic relaxation, tau, which is the gold standard for the LV relaxation velocity. These finding were suggestive of the negative lusitropic potential of nifekalant. Although there have been no reports of compromising data regarding hemodynamic changes induced by nifekalant in patients with acute extensive infarction, severe ventricular dysfunction, or ventricular tachycardia/fibrillation [21, 27], negative lusitropy was a significant side effects induced by medicines [22]. Myocardial relaxation occurs when free intracellular Ca^{2+} concentration decreases due to Ca^{2+} being released from troponin C. Reducing intracellular Ca^{2+} concentration is dependent

Table 2. Percentage change of cardiovascular parameters

Parameters	Animal No.				Mean ± SEM
	No. 1	No. 2	No. 3	No. 4	
C_{max} (ng/mL)	456	319	540	531	462 ± 51
ΔHR (%)	−13	−6	−13	−13	−11 ± 2
ΔLVEDP (%)	+6	+32	+11	+6	+14 ± 6
ΔLVdP/dt$_{min}$ (%)	−42	−18	−15	−31	−27 ± 6
ΔTau (%)	+44	+57	+30	+22	+38 ± 8
ΔQT interval (%)	+34	+54	+59	+25	+43 ± 8
ΔQTcB (%)	+25	+49	+49	+16	+35 ± 8
ΔQTcF (%)	+28	+50	+52	+19	+37 ± 8
ΔQTcV (%)	+28	+46	+50	+19	+36 ± 7

Data represent the maximum percentage changes from their corresponding pre-drug control value after the administration of 0.3 mg/kg of nifekalant. SEM, standard error of the mean; C_{max}, maximum plasma drug concentration; HR, heart rate; LVEDP, left ventricular end-diastolic pressure; LVdP/dt$_{min}$, maximal rate of the fall of left ventricular pressure; Tau, time constant for isovolumic relaxation; QTcB, QT interval corrected by Bazett's formula; QTcF, QT interval corrected by Fridericia's formula; QTcV, QT interval corrected by Van de Water's formula.

on active pumping and uptake of Ca^{2+} by the sarcoplasmic reticulum (SR) or extrusion of cytosolic free Ca^{2+} to the extracellular space by a voltage sensitive Na^+/Ca^{2+} exchange mechanism. It is uncertain whether nifekalant has any influence on the sensitivity of troponin C in terms of Ca^{2+}, SR, or Na^+/Ca^{2+} exchange mechanisms, however, when the cardiac repolarization phase is prolonged by nifekalant, the extrusion of intracellular Ca^{2+} to the extracellular space could theoretically be delayed or reduced, resulting in prolonged Ca^{2+} retention in the cytoplasm [9]. It could be assumed that these changes in C^{2+} kinetics in cardiomyocytes induced by nifekalant were not sufficiently significant as to affect contractile activity, but led to a decreased ventricular relaxation velocity and prolonged the ventricular relaxation phase.

No changes in EDPVR and EDPVR_β were observed after nifekalant administration, which indicated no effects occurred on cardiac compliance (e.g. an increase in myocardial stiffness), suggesting myocardial distensibility might be modulated in spite of negative lusitropic action by nifekalant [8]. These effects would be related to the fact that little side effects due to negative inotropy have been reported for nifekalant use in clinical. Heart rate was decreased after nifekalant administration by its I_{Kr} inhibitory effects, accompanied with QT/QTc prolongation [12, 25]. Nifekalant increased LVEDP, which is suggestive of an increase in preload. The phar-

macological activity of nifekalant was observed as a prolongation of QT/QTc by a decrease in potassium conductance via its I_{Kr} inhibition [12, 25]. In addition, higher doses of nifekalant induced ventricular arrhythmia in 3 of 4 monkeys (Animal Nos. 1, 2, and 3). Plasma drug concentration was not particularly different among the animals, and it was uncertain why 1 monkey (Animal No. 4) did not show arrhythmia from the parameters examined in this study. While, these findings were good accordance with a previous report using a chronic AV block dog model treated with a 10-fold higher than clinically recommended dose of nifekalant that showed a TdP with remarkable QT/QTc prolongation [24]. The EMw has been proposed as a promising and reliable index for proarrhythmic risks in the anesthetized dog [29]. In this study, the EMw was shortened to negative after nifekalant administration. However, the proarrhythmic index of EMw was elusive because similar changes were observed in the isoflurane-anesthetized monkey treated with nicorandil [15], which has no reported arrhythmogenic potential in either non-clinical or clinical stages. The other proarrhythmic indicators, STV and LTV [26], were higher in 3 out 4 animals showing arrhythmia was induced by nifekalant compared the other animal without arrhythmia. It should be noted that nifekalant induces a higher ratio of ventricular tachycardia, including TdP, as described in the packaging insert

of nifekalant, in comparison with other Class III anti-arrhythmic drugs available in clinical practice [23].

Individual analysis for percentage changes of the hemodynamic and electrophysiological effects of nifekalant are summarized in Table 2. Although little individual differences in the extent of the percentage changes in the HR and QT/QTc were observed, hemodynamic effects, including LVEDP, $LVdP/dt_{min}$ and tau, showed a little different values among the individuals. Furthermore, there were no clear correlation between the extent of the percentage changes in the cardiovascular parameters and maximum plasma drug concentration (C_{max}).

In conclusion, the present study identified the negative lusitropic potency of nifekalant at therapeutic doses as a new variable of its cardiovascular activity, based on the LV PV loop analysis in cynomolgus monkeys, in addition to its well-known proarrhythmic potential accompanied by QT/QTc prolongation at high doses. The findings in this study provide useful information suggesting that it may be necessary not only to monitor QT/QTc by ECG but also to monitor lusitropic activity if nifekalant is to be used safely in patients.

Conflict of Interest

Acknowledgments

References

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