

Original article

# A novel propellant-free inhalation drug delivery system for cardiovascular drug safety evaluation in conscious dogs

Michael Markert\*, Anja Klumpp, Thomas Trautmann, Brian Guth

Department of Drug Discovery Support, General Pharmacology Group, Boehringer Ingelheim Pharma GmbH & Co KG, Germany

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## Abstract

**Introduction:** Estimation of possible cardiovascular side effects belongs to the safety assessment of every drug candidate. This paper describes a new strategy for treating conscious labrador dogs with drugs by inhalation using a specially designed mask and a novel inhaler device. **Methods:** Labrador dogs (male or female) were used that had transducers implanted for the measurement of left ventricular and descending aortic blood pressures and an ECG for use together with a telemetry system. Administration by inhalation was achieved using a novel delivery device. The Respimat device is a propellant-free inhaler to deliver aerosols from solutions. The new system was evaluated using Formoterol with four dogs using a 4 × 4 Latin square design. Three doses of Formoterol (0.6, 1.2, and 2.4 µg/kg, dissolved in 60% ethanol) were administered by inhalation together with a vehicle (60% ethanol) treatment by applying three inhalations, each consisting of 10 µl solution. **Results:** Formoterol increased HR, QRS-interval, QT-interval, and LVPdP/dtmax and dose-dependently decreased systolic and diastolic BP. This effect lasted up to 14 h. **Discussion:** Drug administration by inhalation in the conscious labrador dog using the Respimat is a useful new model for safety pharmacology studies of new drug candidates that are intended to be given by inhalation in the clinic. © 2004 Elsevier Inc. All rights reserved.

**Keywords:** Methods; Conscious labrador dog; Respimat; QT-interval; By inhalation; Long-acting β<sub>2</sub>-adrenoceptor agonist

## 1. Introduction

Estimation of possible cardiovascular side effects belongs to the safety assessment of every drug candidate. The model preferred for such tests in the ICH S7a guideline (FDA, 2001) is the conscious dog, because possible effects of anesthetic agents are avoided. Also mentioned in the ICH S7a guideline, drugs should be administered using the intended clinical route. In the conscious dog, oral, intravenous, and subcutaneous routes are easily accessible. Drugs meant to be administered by the inhalation route, however, are often tested under general anesthesia and/or after surgical intervention (tracheotomy), due to the difficulty of administering drugs by inhalation to the conscious dog. This paper describes a new strategy for treating conscious labrador dogs with drugs by inhalation using a specially designed mask and a novel inhaler device. The conscious dog is a good model because it is a large animal species with lung deposition characteristics similar to man (Cuddihy,

Brownstein, Raabe, & Kanapilly, 1973; Wolff, Kanapilly, Gray, & McClellan, 1984). With the telemetry system used, the cardiovascular parameters could even be acquired and analyzed during the inhalation.

## 2. Methods

### 2.1. Animals

Trained labrador dogs (male or female), at least 1 year of age with body weights between 22 and 30 kg, bred at Boehringer Ingelheim Pharma, Biberach, were used. The dogs were group housed as pairs of two in separate cages and had access to water ad libitum and were fed a standard dog diet once daily. They also had daily exercise periods of at least 1 h, each afternoon.

### 2.2. Materials

#### 2.2.1. Respimat

The Respimat device (Fig. 1) is a novel propellant-free inhaler to deliver aerosols from solutions. The aerosol is

\* Corresponding author.

E-mail address: [Michael.Markert@bc.boehringer-ingelheim.com](mailto:Michael.Markert@bc.boehringer-ingelheim.com) (M. Markert).

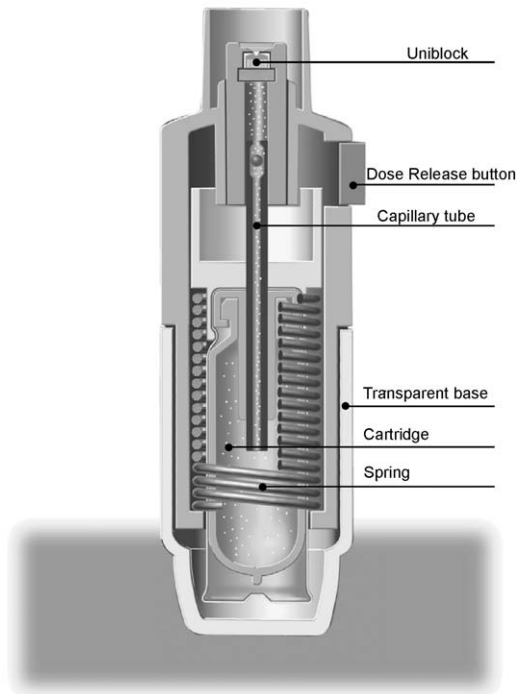


Fig. 1. The Respimat device.

generated by pressing a premeasured amount of liquid through a filter/nozzle system to form two jets disintegrating into fine droplets after leaving the nozzle (Zierenberg, 1999). The basic principle of the purely mechanical inhaler is to press an exactly measured dose of the active substance through a microdosage nozzle using high pressure. The energy from 180° twist of the base compresses a spring and draws a predefined, metered volume of solution. When the dose-release button is pressed, the energy released forces the solution through the nozzle system at a pressure of around 250 bar. On the two outlets, two fine liquid jets are generated, which collide with each other. In doing so, they form a very fine and long-lasting liquid aerosol, which is exhausted from the mouthpiece (Fig. 2). A micropump ensures even dosing of the preparation. A special advantage of the Respimat is that it requires no propelling gas, and the utilised active nebulising principle also significantly increases the efficiency of the inhaler. The patient often requires only a fraction of the amount of a drug delivered conventionally. Clouds generated by the Respimat inhaler are considerably slower and more prolonged than those delivered by either CFC or HFA MDIs. These cloud characteristics demonstrate that the Respimat inhaler is a soft mist inhaler, the use of which results in improved lung and reduced oropharyngeal deposition (Hochrainer & Hölz, 2001). It meters accurate doses (10 µl). In vitro assessment of aqueous clouds showed a fine particle size of 5 µm, and for ethanolic solutions, clouds are even finer. These clouds are generated with a low velocity (<10 m/s)

over a comparatively long period (1.5 s). This facilitates coordination of dose delivery in a slow-breathing maneuver designed to give a good lung penetration. The advantage of a fine slow-moving cloud are shown in preliminary clinical evaluations, where 12.5 or 25 µg of fenoterol delivered by the Respimat was therapeutically equivalent to 100 or 200 µg administered by a pMDI (Ganderton, 1999).

In scintigraphic studies, it was shown unequivocally that the Respimat delivers a much greater percentage of drug doses to the lungs than a pMDI, irrespective of whether the Respimat was used to deliver an aqueous-based formulation or an ethanolic solution. The Respimat increased lung deposition and reduced oropharyngeal deposition at the same time, thereby targeting the drug to the required site of action in the lungs better than a pMDI (Newmann, 1999). Because the Respimat doubles the amount of drug deposited in the lungs, it is to be expected that inhaled asthma drugs will maintain their efficacy when the dose is reduced. Clinical data showed this to be the case for the Respimat (Vincken et al., 1997).

#### 2.2.2. Inhalation mask

A mask was specially designed for the dogs because no commercially available mask could be found. We developed a plastic mask that was connected to a specially designed tubing system that could be connected to the Respimat device (Fig. 3). To get an increased lung deposition, we used a wide delivery tubing, thus reducing wall losses and the end of the tube was shaped to seal the trachea. The mask has an integrated tube that extends into the dog's mouth. The nostrils are closed by an elastic rubber stopper inside the mask, so that the dogs can only breathe through the mouth. There is a small balloon attached to the tube which is used as an indicator for in-/expiration. This is used to ensure that the dose-release



Fig. 2. The Respimat device.

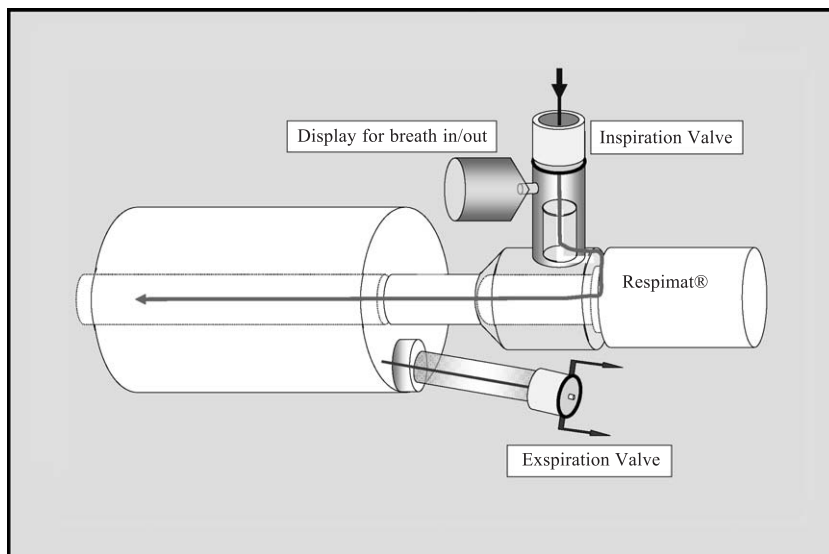


Fig. 3. The inhalation mask.

button of the Respimat is pressed exactly when the dog is breathing in.

### 2.2.3. Formoterol

Formoterol is a long-acting  $\beta_2$ -adrenoceptor agonist which is used as a bronchodilator agent in asthma (Bartow & Brogden, 1998; Beasley, Crane, & Burgess, 2000; Moore, Khan, & Dickey, 1998; Redington & Rees, 1998). Formoterol has a rapid onset of action and is an effective bronchodilator for at least 12 h (Tattersfield, 1993). Formoterol (formoterol fumarate, synthesized at Boehringer Ingelheim Pharma) was dissolved in 60% ethanol for these studies.

### 2.2.4. Telemetry system

The telemetry system used to measure cardiovascular parameters is manufactured by Konigsberg Instruments, (Pasadena, CA) and marketed by RMISS (Delaware). It consists of five major components: (a) an implantable unit; (b) a receiver (antenna) located in the animals' cage together with an amplifier; (c) ambient pressure monitor to measure atmospheric pressure; (d) a PC-based "base station" to receive and process the amplified signals; and (e) a PC-based data acquisition system (NOTOCORD Hem 3.5) to process signals.

The implantable unit ("T27" total implant) consists of (1) two high-fidelity pressure transducers (5.0 and 4.0 mm diameter), (2) ECG cable, (3) micropower battery-operated electronics that process and digitize the information from the pressure transducers and the ECG lead, (4) a radio-frequency transmitter that sends the signals to the telemetry receiver, and (5) a battery. A small cable projecting from the transmitter contains a magnetic switch that allows the device to be turned on and off. Prior to implantation, the zero value

of the two pressure transducers is calibrated using a manometer and 250 mm Hg is set to 5 V.

### 2.3. One-time surgical implantation

The transducers of the T27 implant were calibrated and the unit was sterilized using a low-pressure ethylene oxide process prior to implantation.

Dogs were anaesthetised with a combination of Rompun (xylazine hydrochloride, 1 ml/10 kg iv) and Ketavet (ketamine hydrochloride, 0.7 ml/10 kg iv) after premedication with Atropine (0.04 mg/kg im) and ventilated with 33% N<sub>2</sub>O and 66% O<sub>2</sub> and 1% Isoflurane. All procedures were performed under aseptic conditions using sterilized equipment.

The dogs were placed in a lateral recumbency with the left side facing the surgeon. An incision was made between the fifth and sixth intercostal spaces. A small pocket was opened in the abdominal wall for implantation of the transmitter, battery housing, and induction switch coil. The cables with both pressure transducers and ECG leads extending from the ventrally implanted transmitter were guided subcutaneously to the lateral incision. The antenna was guided subcutaneously from the transmitter location towards the spine and then runs parallel to the spine for approximately 25 cm. The initial ventral incisions required for battery and transmitter placement were closed.

A left thoracotomy was performed between the fifth and sixth intercostal spaces to expose the left ventricle apex for insertion of the left ventricular Konigsberg transducer. The aorta pressure transducer was implanted next. The aortic transducer, which also served as one electrode of the ECG, was inserted into the thoracic aorta

Table 1

Formoterol: dependent variable RR; *P* values for the ANOVA and for the one-sided comparisons between control and Formoterol-treated groups

Treatment	Observation time (min) after administration									
	10	30	60	120	180	240	300	360	420	600
overall <i>F</i> value (ANOVA)	0.6413	0.1651	0.2074	0.1889	<b>0.0473</b>	0.1081	0.1356	<b>0.0318</b>	0.2965	0.2554
0.6 µg/kg Formoterol	0.5838	0.1135	<i>0.0878</i>	0.3054	0.1641	0.2750	0.1434	0.5355	0.1907	0.5661
1.2 µg/kg Formoterol	0.3206	0.0276	<b>0.0272</b>	<b>0.0467</b>	<b>0.0115</b>	<b>0.0308</b>	<b>0.0328</b>	<b>0.0221</b>	<i>0.0528</i>	0.1273
2.4 µg/kg Formoterol	0.2391	0.0261	<b>0.0352</b>	<i>0.0565</i>	<b>0.0121</b>	<b>0.0315</b>	<b>0.0205</b>	<b>0.0319</b>	<i>0.0582</i>	0.1251

All significant *P* values ( $\leq 0.05$ ) are marked bold, indicative *P* values ( $0.05 < P \leq 0.1$ ) are set in italics.

just below the aortic arch. The transducer was sutured into place and blood flow was restored. Chest incisions were closed. The lung was then inflated and the intercostal muscles were sutured closed and the pneumothorax was evacuated.

The gas anaesthesia was then turned off and dogs were allowed to wake up. Analgesics and antibiotics (Temgesic and Tardomycel, Benzylpenicillin-benzatin and Benzylpenicillin-procain) were administered for 10 days following the procedure (Temgesic was only administered 1–2 days). Dogs are allowed to recover for at least 14 days before experiments using initiated test substances.

#### 2.4. Experimental design

Experiments were performed with four dogs using a  $4 \times 4$  Latin square design. Three doses of 10 µl of the appropriate Formoterol solution were administered by inhalation together with a vehicle treatment (60% ethanol).

An initial equilibrium period of 30–45 min allowed the dogs to acclimate to the measurement pens and the administration of the test compound was started 120 min after a baseline measurement. After dosing, the dogs were left undisturbed, but were monitored by video for the duration of the study, which lasted 24 h and had access to water ad

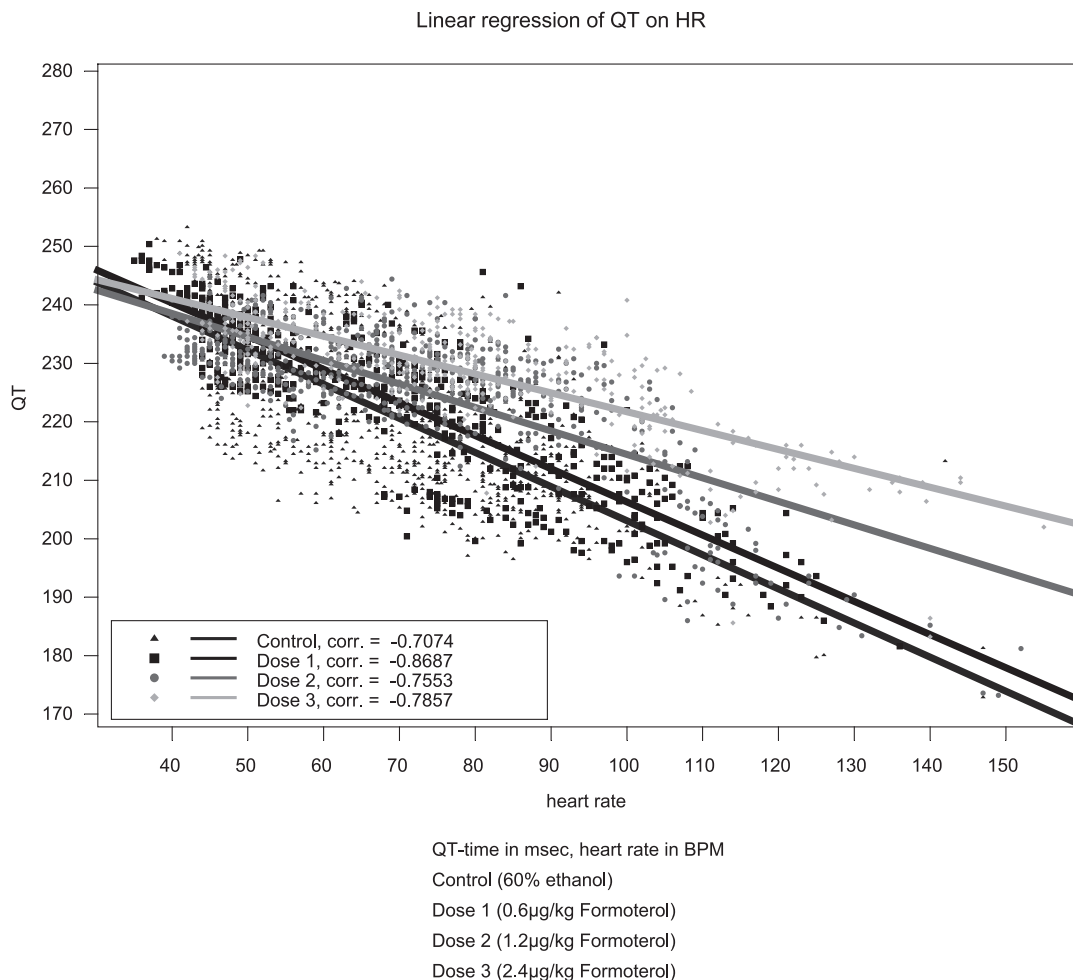


Fig. 4. Effects of Formoterol on uncorrected QT-interval.

libitum (at 7 h after dosing, the dogs were fed with a standard diet).

### 2.5. Data acquisition and analysis

Digitized telemetry signals were processed by NOTOCORD software (Hem 3.5) on a beat-to-beat basis. The following parameters were continuously recorded for the duration of the experiment: aortic pressure (AP), left ventricular pressure (LVP), ECG lead II, and body temperature. The hemodynamic and ECG parameters were calculated during the experiment and include the following: systolic, diastolic, and mean AP, peak systolic and end-diastolic LVP, LV dP/dt max and dP/dt min, heart rate, PQ-, QRS-, and QT-intervals. NOTOCORD software was used for acquisition of data whereas EXCEL was used for some basic data analyses. Data were summarized at predefined time points by calculating median values + S.D. At each time point, a minimum of 400 sequential beats was used to calculate the median value.

The summarized data are given as mean values + S.E.M. The different base levels of the individual animals

were taken into account by referring the measured values after administration of the test compound to the pretreatment values. With these values, the standardised area under the curve (AUC divided by interval length) was calculated for the time intervals of interest. Comparisons between treatment and placebo were performed by one-way analysis of variance (ANOVA) followed by the Student's *t* test. Statistical significance was accepted when  $P < .05$ . Using the error term of the ANOVA as an estimate for the variability, many-to-one *t* tests comparing the dose groups of the test compound with the control group were added (Table 1). The evaluation was performed using the software package SAS 8.02 (SAS Institute, Cary, USA).

For the correction of the QT–RR relationship, the Sarma equation was used (Sarma, Sarma, Bilitch, Katz, & Song, 1984).

Correction formulas were based on the results of a previous study of QT correction on labrador dogs. Using the observed values of RR and QT, respectively, the following different corrections have been determined according to the following models:

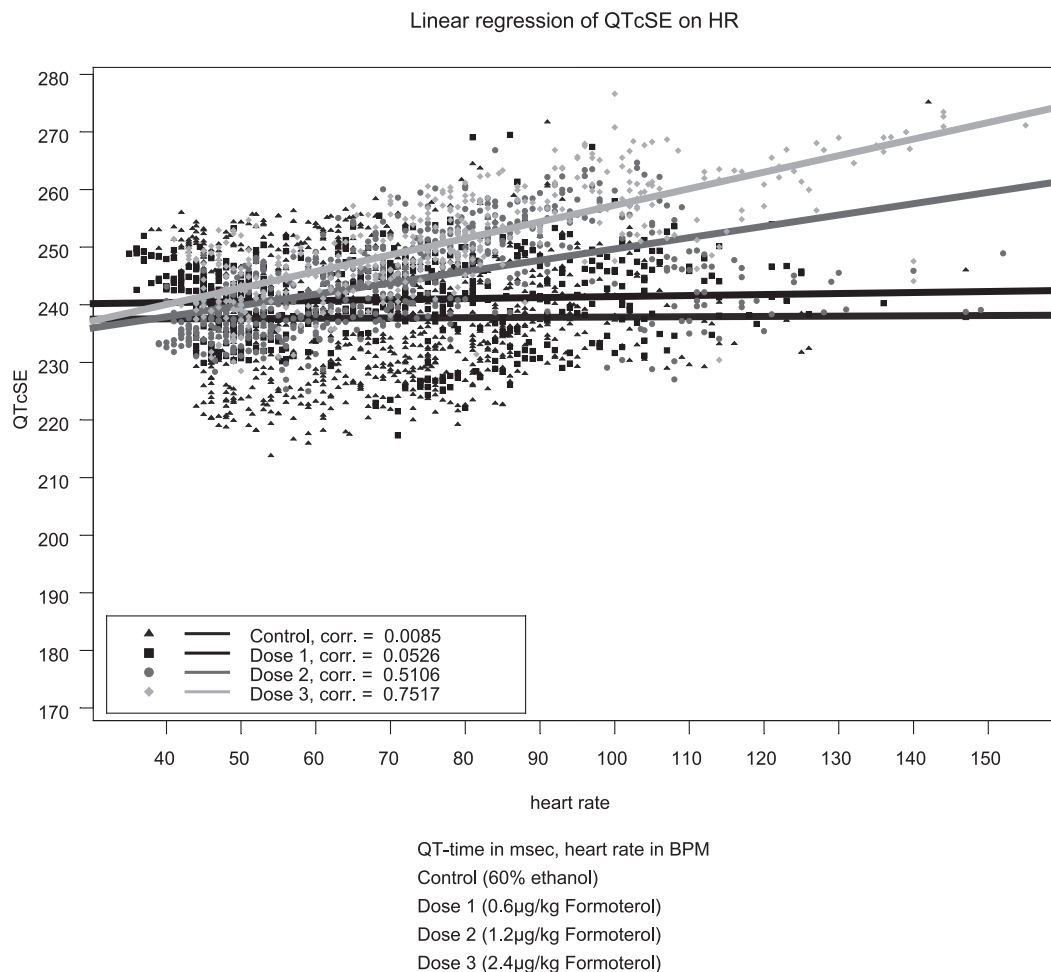


Fig. 5. Effects of Formoterol on Sarma-corrected QT-interval.

The transformation according to Sarma, using parameters estimated from a prior study on untreated control animals:  $QT_{cSE} = QT + 230 \times \exp(-3.11 \times RR)$ .

Additionally, transformations according to three models considered by Malik et al. (Heart 87, 2002), also with parameter estimates from the abovementioned prestudy 309:

- Model A—linear model:  $QT_{cMA} = QT + 35.13 \times (1 - RR)$
- Model C—parabolic model:  $QT_{cMC} = QT/RR^{0.15}$
- Model D—logarithmic model:  $QT_{cMD} = QT - 36.45 \times \log(RR)$

From these values, the standardized AUC (divided by interval length) was calculated. Here, two phases have been considered, while the specification of the time intervals was as follows:

- 10 min until 13 h after administration of the treatment,
- 13–24 h after administration of the treatment.

An ANOVA for crossover studies was considered with the factors dose and animal. Using the error variance  $\sigma$  of the ANOVA as an estimate for the variability, many-to-one  $t$  tests comparing the different dosage groups with vehicle control were considered. The least-squares means are reported as well. The significance level  $\alpha$  for the comparisons was set to .05.

In addition, the power for the many-to-one tests is reported. To determine the power, a relevant difference  $d$

of interest had to be defined. Here,  $d=1, 5, 10$ , and 20 percentage points had been considered, respectively. A relevant difference for QT had been agreed to be 10 percentage points. The respective values for each parameter are marked bold in the result tables, while the other values are reported for descriptive purposes only.

To illustrate the results, the observations of QT/QTc are plotted against HR and the respective correlations are determined and graphically displayed as the corresponding regression line (Figs. 4 and 5).

The statistical evaluation was prepared using the software packages SAS Version 8.2 and S Plus Version 6.1.

### 3. Results

Formoterol significantly increased HR, QRS-interval, QT-interval (corrected with the Sarma equation), and LV dP/dtmax and decreased systolic and significantly diastolic BP as described by other authors (Bremner et al., 1993; Flatt et al., 1990) which lasted up to 14 h.

#### 3.1. Systolic blood pressure (SBP)

There was a dose-dependent decrease in SBP (Fig. 6). With the lowest dose (0.6  $\mu\text{g}/\text{kg}$ ), a 10% decrease in SBP (peak at 2 h postdose) was observed, which lasted about 6 h. The change in SBP within the medium dose (1.2  $\mu\text{g}/\text{kg}$ ) decreased the blood pressure up to 15% (peak at

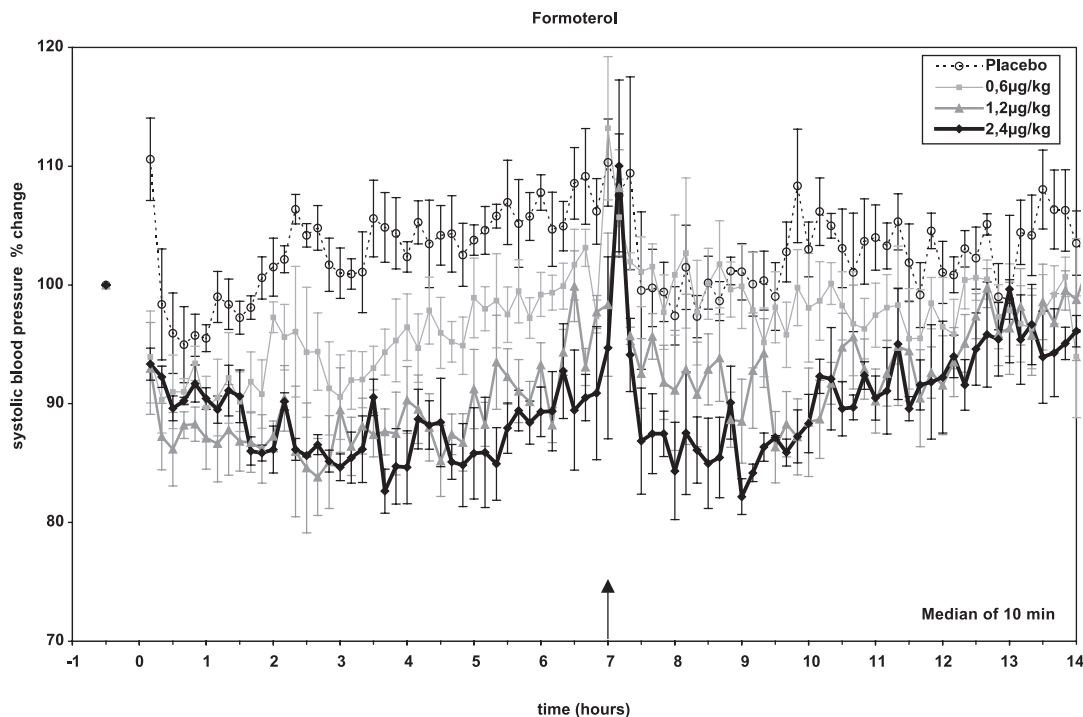


Fig. 6. Effects of Formoterol on SBP (percent change from prevalue). The arrow indicates the effects of feeding the dogs.

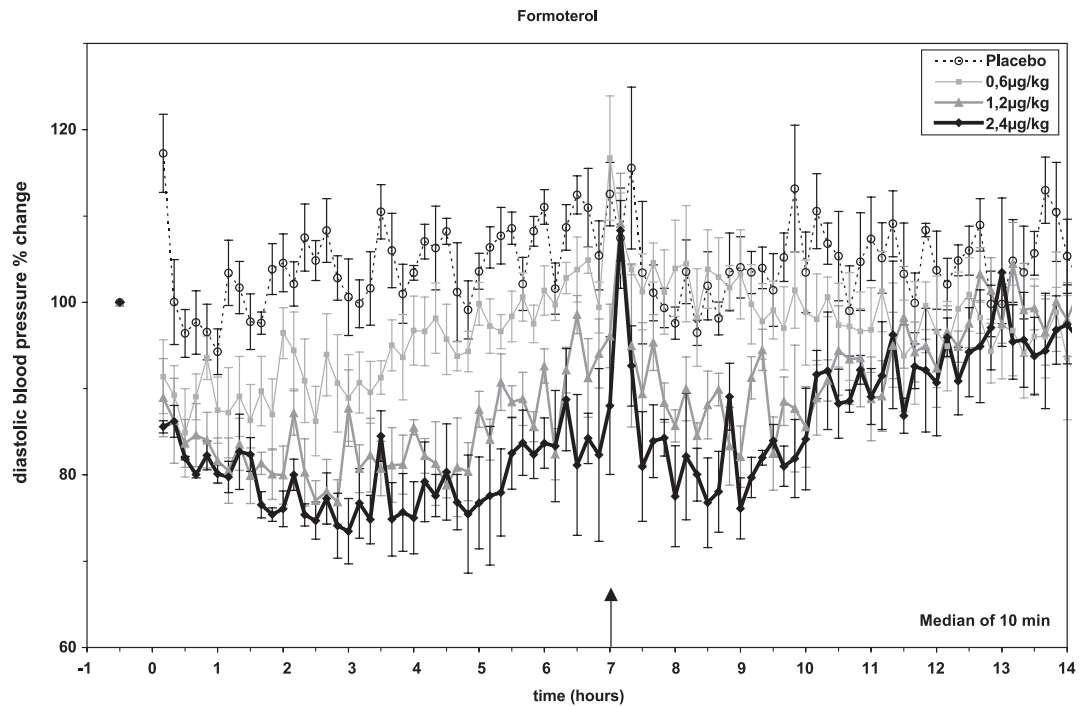


Fig. 7. Effects of Formoterol on DBP (percent change from prevalue). The arrow indicates the effects of feeding the dogs.

2.5 h after dosing) from baseline values. This decrease in SBP lasted up to 13 h until it reached baseline values again. With the highest dose (2.4  $\mu\text{g}/\text{kg}$ ), there was a similar decrease of 15% (peak at 2.5 h after dosing) of pre-dose value which lasted up to 13 h post-dosing.

### 3.2. Diastolic blood pressure (DBP)

There was a dose-dependent decrease in DBP (Fig. 7). With the lowest dose (0.6  $\mu\text{g}/\text{kg}$ ), a 14% decrease in DBP (peak at 2 h post-dose) was observed, which lasted about 6 h.

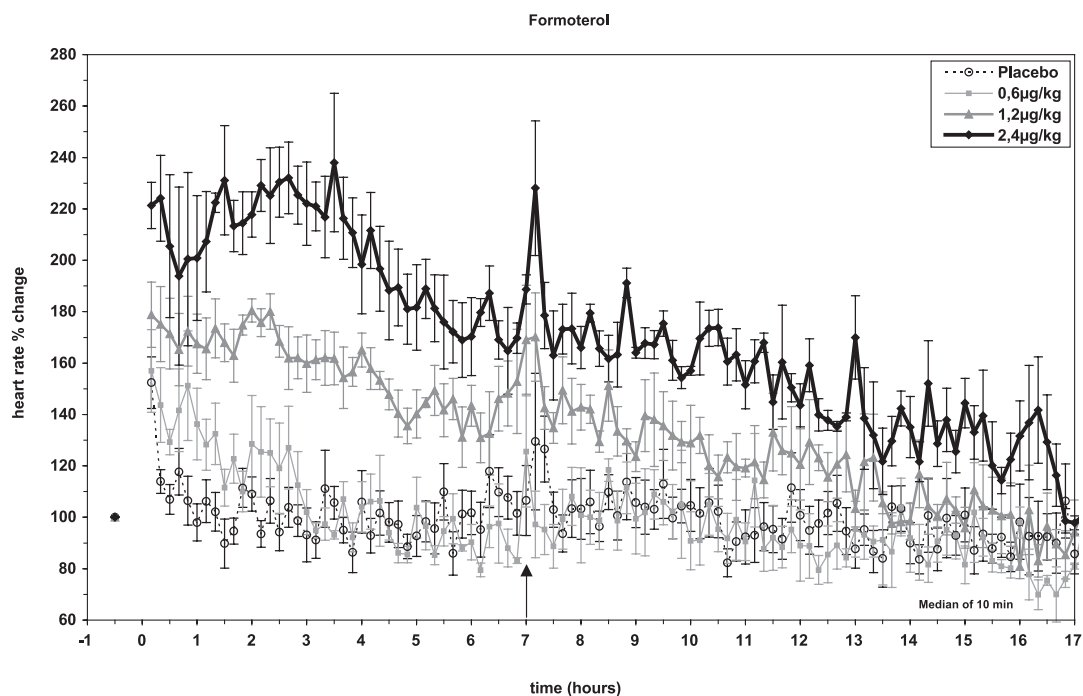


Fig. 8. Effects of Formoterol on heart rate (percent change from prevalue). The arrow indicates the effects of feeding the dogs.

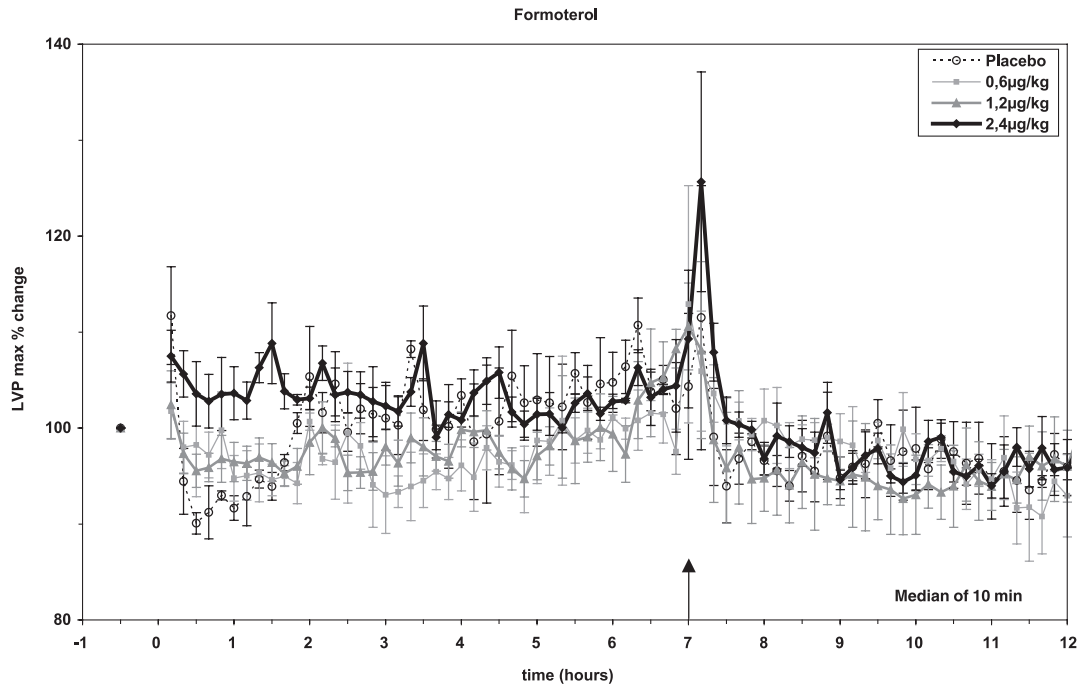


Fig. 9. Effects of Formoterol on LVPmax (percent change from prevalue). The arrow indicates the effects of feeding the dogs.

A significant ( $P < .05$ ) decrease was observed in DBP within the medium dose (1.2 µg/kg) up to 23% (peak at 2.5h after dosing) from baseline values. This decrease in DBP lasted up to 13 h. With the highest dose (2.4 µg/kg), there was a significant ( $P < .05$ ) decrease of 26% (peak at 3 h after dosing) of predose value which lasted up to 13 h postdosing.

### 3.3. Heart rate

There was a dose-dependent increase in heart rate (Fig. 8). With the lowest dose (0.6 µg/kg), a slight increase in heart rate was observed, which lasted about 1 h. With the medium dose (1.2 µg/kg), there was a significant ( $P < .05$ ) change in heart rate up to 80% (peak at 2 h after dosing) from baseline values.

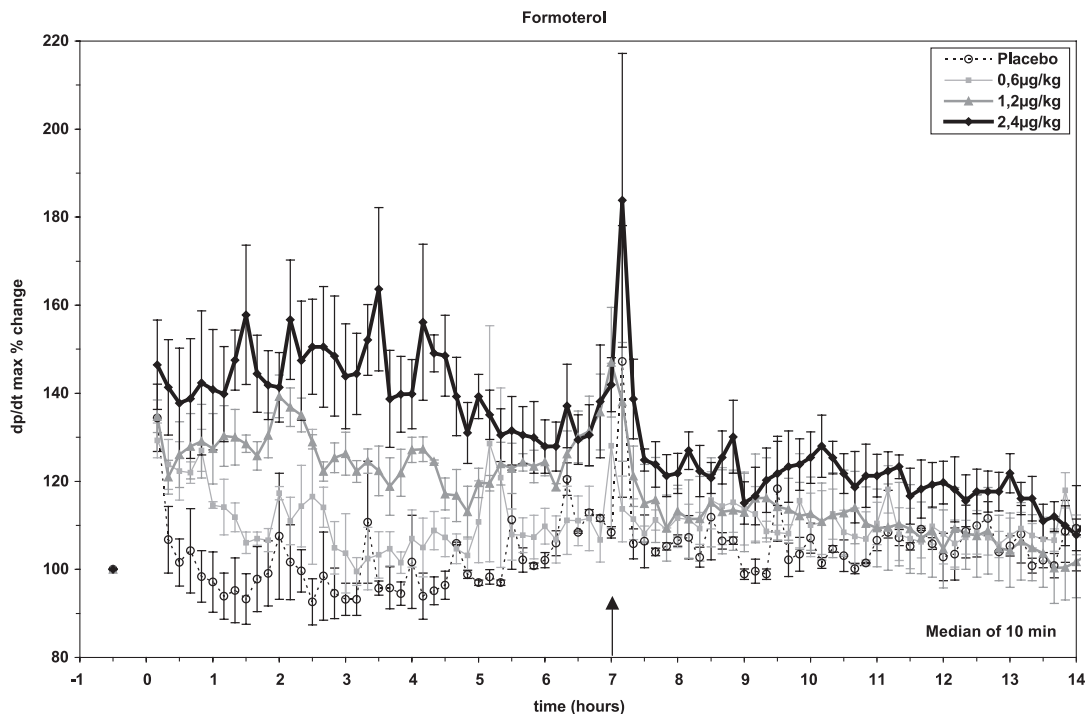


Fig. 10. Effects of Formoterol on LVPdp/dtmax (percent change from prevalue). The arrow indicates the effects of feeding the dogs.

This increase in heart rate lasted up to 13 h until it reached baseline values again. With the highest dose (2.4 µg/kg), there was a marked, highly significant ( $P < .01$ ) increase in heart rate up to 130% (peak at 2.5 h after dosing) of the predose value which lasted up to 13 h postdosing.

#### 3.4. Maximum left ventricular pressure (LVPmax)

There was a slight increase of 10% (peak at 1.5 h after dosing) in LVPmax only with the highest dose (2.4 µg/kg), which lasted up to 1 h postdosing (Fig. 9).

#### 3.5. LV dP/dtmax

There was a dose-dependent increase in LV dP/dtmax (Fig. 10). With the lowest dose (0.6 µg/kg), a 22% increase in LV dP/dtmax (peak at 1 h postdose) was observed, which lasted about 3 h. A significant ( $P < .05$ ) increase in LV dP/dtmax was seen with the medium dose (1.2 µg/kg) up to 39% (peak at 2 h after dosing) from baseline values. This increase lasted up to 7 h. With the highest dose (2.4 µg/kg), there was a significant ( $P < .05$ ) increase of 64% (peak at 3.5 h after dosing) of predose value which lasted up to 10 h postdosing.

#### 3.6. QRS-interval

There was an increase of 30% (peak at 2h after dosing) in the QRS-interval only in the highest dose (2.4 µg/kg), lasting up to 3 h postdosing.

Table 2

Corrected QT-interval

	Placebo	Formoterol		
		0.6 µg/kg	1.2 µg/kg	2.4 µg/kg
	100.426	98.249	104.270	112.079
	100.576	103.315	105.356	106.541
	96.313	103.214	106.244	105.828
	99.478	101.421	102.819	
	100.293			
	100.235			
N	6	4	4	3
Mean	99.553	101.550	104.672	108.149
S.D.	1.632	2.366	1.476	3.422
LS means	101.031	100.448	103.658	106.016
P value		.7589	.1956	.0520
Power ( $\delta = 1$ )		0.1443	0.1443	0.1202
Power ( $\delta = 5$ )		0.9922	0.9922	0.9673
Power ( $\delta = 10$ )		1.0000	1.0000	1.0000
Power ( $\delta = 20$ )		1.0000	1.0000	1.0000

QT<sub>cSE</sub> [percentage of prevalue]; standardized AUC values; 10 min–13 h after administration of the test compound.

#### 3.7. QT-interval

Using the data uncorrected for heart rate, there was a slight increase in QT-interval with the lowest dose (0.6 µg/kg; 3%, peak at 3.5 h postdose), which lasted about 6 h. With the medium dose (1.2 µg/kg), QT-interval was not changed. With the highest dose (2.4 µg/kg), there was a decrease of 10% (peak at 3.5 h after dosing) of predose value, which lasted up to 4 h postdosing (Fig. 11).

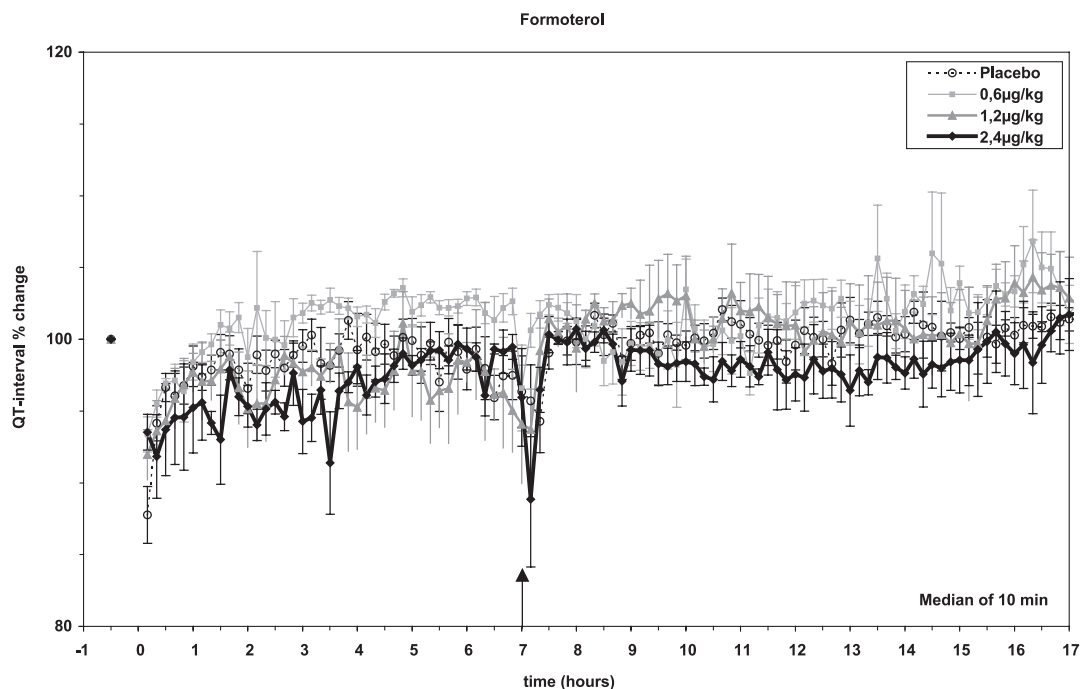


Fig. 11. Effects of Formoterol on uncorrected QT-interval (percent change from prevalue). The arrow indicates the effects of feeding the dogs.

Because the heart rate was not affected in the lowest dose, this means that there was a slight QT-interval prolongation at that dose. In the medium and highest dose, there was an 80–130% increase in heart rate and no change in QT-interval; this means that there was a QT-interval prolongation as one would expect a shortening of the QT-interval. The linear regression approach (and hence, the correlation) was shown not to be adequate for the Malik models: the residuals did not seem to be independent, rather a quadratic relationship was indicated. Using the correction according to the Sarma model  $QT_{cSE}$ , there was a prolonging effect of the two higher doses on QT at all points in time, as most *P* values were significant or at least suggestive of an effect (Table 2).

#### 4. Discussion

The purpose of this study was to establish the feasibility of using a novel inhaler to administer drugs by inhalation to conscious labrador dogs. We used the new Respimat® device with a specially developed mask to assess the cardiovascular drug action of application by inhalation of Formoterol. After a short training period, the dogs accepted the mask very well. The results indicate that this is an attractive system for the application of drugs by inhalation.

To validate this novel application form, we used Formoterol, a long-acting  $\beta_2$ -agonist with well-known cardiovascular effects. Because the applied doses of Formoterol were low, no pharmacokinetic blood sample analysis could be performed. We used instead, for showing the sensitivity of the model, the dose–response relationship to the cardiovascular effects of Formoterol. In our conscious dog model, Formoterol significantly increased HR, QRS-interval, QT-interval (corrected with the Sarma equation; Sarma, Sarma, Bilitch, Katz, & Song, 1984), and LV  $dP/dt_{max}$  and decreased systolic and diastolic BP as described by other authors (Bremner et al., 1993; Flatt et al., 1990; Cazzola et al., 1998). These effects were shown to last up to 14 h.

The main adverse side effects of inhaled long acting  $\beta_2$ -agonists relate to their systemic activity. Within minutes of inhalation, Formoterol caused an increase in heart rate and a fall in DBP, suggesting that the drug was absorbed rapidly and was available systemically even with administration by inhalation. This has been reported in previous studies, in which peak systemic plasma levels of Formoterol occurred within 5 min of inhalation in normal subjects (Lecaillon, Kaiser, & Palmisano, 1999). The early tachycardia has been shown in previous studies (Guhan, Cooper, Osborne, Lewis, Bennett & Tattersfield, 2000) and is likely to be caused by a direct effect on cardiac  $\beta_2$ -receptors (Brown, McLeod, & Shuad, 1986; Strauss, Reeves, & Smith, 1986). The decrease of DBP has been reported in some previous studies (Bennett & Tattersfield, 1997; Bremner et al., 1993; Guhan et al., 2000) and is caused by  $\beta_2$ -mediated dilatation of peripheral arterioles.

This decrease in peripheral vascular resistance may also contribute to the tachycardia through a reflex increase in heart rate. The described effects on the cardiovascular system are expected with the application of long-acting  $\beta_2$ -agonist. It is generally accepted that in the human heart and possibly in the canine heart, functional  $\nu_1$ - and  $\beta_2$ -adrenoceptors coexist (Brodde et al., 1986). Both  $\beta_1$ - and  $\beta_2$ -adrenoceptors can mediate positive inotropic and chronotropic effects through increasing the intracellular amount of cyclic AMP. In the human heart, the functional  $\beta_1/\beta_2$  ratio is approximately 65:35% for the atria and 75:25% for the ventricles (Brodde et al., 1986). Because Formoterol has only a “relative” selectivity for  $\nu_2$  receptors (about 30-fold), one could argue that the described effects are a combination of the stimulation of both  $\beta$ -adrenoceptor subtypes. In the sinus node,  $\beta_2$ -adrenoceptor stimulation increases the slope of the slow diastolic depolarization and maximum diastolic potential as well as accelerates repolarization (Vaughan Williams, 1985). Furthermore,  $\beta_2$ -adrenoceptor stimulation accelerates repolarization in Purkinje cells and papillary muscle.

There were morphological changes in the T-wave (flattening and bifurcation of the T-wave, interruption of the ascending limb) of the ECG with the highest and medium dose of Formoterol. These effects were pronounced at the maximum heart-rate effect (4 h after application) but disappeared after 13 h. Because it is known that Formoterol and other long-acting  $\beta_2$ -agonists lower serum potassium levels, this effect on T-wave morphology might be due to low  $[K^+]_o$ .  $\beta$ -Agonist can decrease potassium serum levels by stimulation of  $\beta_2$ -adrenoceptors in the liver and skeletal muscle through an increase in the transport of potassium ions into cells by activation of  $Na^+ - K^+$  adenosine triphosphatase (Clapham & Hamilton, 1992). Cazzola et al. (1998) also showed an increase in isolated supraventricular premature beats in 12 patients after 12  $\mu g$  Formoterol. These observations were absent in the present study.

The QT-interval is heart rate dependent. Because Formoterol led to a substantial tachycardia, this effect needs to be taken into account when assessing the QT-interval duration. There have been numerous formulas used in the past (Carlsson, Abrahamson, Andersson, Duker, & Schiller-Linhardt, 1993), but we found that linear correction formulas did not fit our data. Therefore, the data were fitted using a multicoefficient formula as developed by Sarma et al. (1984). This formula has been shown previously to provide a good fit for canine data (Matsunaga et al., 1997). With this analytic approach, a QT-prolonging effect is clearly seen with Formoterol. Furthermore, the effect appears also to be use dependent with a larger prolongation of the QT-interval at higher heart rates than at lower rates. A dose dependency of this effect was also observed.

Despite the clear effect on QT-interval duration seen in this study, Formoterol has not been associated with torsade de pointes arrhythmias.

## 5. Conclusion

The Respimat may become a useful tool for safety-pharmacological studies when an administration by inhalation is desired. With its use, drugs may be given by inhalation drugs to conscious dogs without resorting to sedation. The high fine-particle fraction, together with the slow and longer-lasting soft mist is the key to its innovative clinical profile (Van Noord, 2000).

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