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Respiratory safety pharmacology: Positive control drug responses in Sprague–Dawley rats, Beagle dogs and cynomolgus monkeys

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ABSTRACT

Rats are most frequently used to fulfill ICH S7A requirements for respiratory safety pharmacology. We hypothesized that the models used to assess respiratory safety pharmacology present different ventilatory responses to bronchoconstriction, bronchodilation and respiratory depression. Respiratory monitoring was performed with head-out plethysmographs for rats, masks for dogs and bias airflow helmets for monkeys. Respiratory rate (RR), tidal volume (TV) and minute volume (MV) were recorded. Forty rats, 18 dogs and 8 monkeys were acclimated to the respiratory monitoring equipment. Animals received saline (IV), albuterol (inhalation), methacholine (IV) and remifentanil (IV). Albuterol increased TV in all species. Methacholine decreased TV and MV in monkeys. In dogs, methacholine increased TV, RR and MV. In rats, methacholine increased TV and decreased RR. Remifentanil induced central respiratory depression in all species with decreased MV, except in rats. Dogs presented a biphasic response to remifentanil with hypoventilation followed by delayed hyperventilation. The monkeys presented similar responses to humans which may be due to biologic similarities. Dogs and rats presented clinically significant ventilatory alterations following positive control drugs. Although, the response to bronchoconstriction in dogs and rats was different from humans, the two species presented ventilatory changes that highlight the potential adverse effect of test articles.

1. Introduction

The ICH S7A guideline defines the safety pharmacology core battery including cardiovascular (CVS), central nervous system (CNS) and respiratory (U.S. Food and Drug Administration, 2001). The guideline states that "respiratory rate and other measures of respiratory function (e.g. tidal volume or hemoglobin oxygen saturation) should be evaluated". A recent industry survey revealed that respiratory rate, tidal volume and minute volume are included in 98–100% of core battery and supplemental respiratory safety pharmacology studies (Lindgren et al., 2008). Study design in safety pharmacology is constantly evolving prodded by emerging technologies (Hoymann, 2007; Murphy et al., 1998, 2001) and integrated drug development where *in vitro*, preclinical and clinical safety testings share a common goal, drug approval. Sensitivity of the model should be commensurate with the risk in our drug development industry where resources are scarce and time is felt by drug companies with aging drug patents. Blood gases, reported to have limited sensitivity (due to complex compensatory mechanisms) to detect drug-induced adverse effects (Authier et al., 2008), and RR are occasionally used as sole *in vivo* markers of preclinical respiratory safety. With the addition of histopathological assessments, these two *in vivo* biomarkers (blood gases and RR) are, in some cases, considered acceptable for respiratory safety testing (e.g. some oncology indications).

When non-clinical and clinical reasons for drug development discontinuation are combined, respiratory adverse effects are less frequent than CVS or CNS (Valentin and Hammond, 2008). Moreover, respiratory safety pharmacology is less frequently frontloaded (performed earlier in the drug development process) than CVS and CNS which may suggest a perceived lower risk of respiratory liability in comparison to the other two systems included in the safety pharmacology core battery.

The rat is used as the preferred species for respiratory safety in most investigational new drug (IND) programs (Lindgren et al., 2008). Advantages to the use of rats are numerous including high genetic homogeneity which reduces variability, lower test material requirements, abundant historical data and ethical considerations which favor the use of a phylogenically lower laboratory animal species. On the flip side, the use of rats may present disadvantages including limited genetic diversity which may not be representative of the human patient population. Respiratory anatomy, physiology and pathology in rats are relatively different from humans,

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an adaptive response to their respective normal habitat. Large laboratory animals such as dogs and monkeys present respiratory system characteristics which could be considered closer to humans.

IND submissions often require that a small and a large animal species be selected for toxicology studies. CVS safety pharmacology assessments are most frequently performed in large animals (Lindgren et al., 2008) using dogs (Gauvin et al., 2006) or monkeys (Authier et al., 2007). Respiratory assessments to fulfill S7A can be performed using minimally invasive methodologies in toxicology studies or in large animals used for CVS safety pharmacology without the need to use additional animals. As large animals present biologic similarities with human patients, inclusion of respiratory measures in large animal studies may increase the clinical relevance of these investigations. The current project presents the ventilatory responses of common respiratory safety pharmacology models to bronchodilation, bronchoconstriction and respiratory depression using well-characterized pharmaceutical agents.

2. Materials and methods

2.1. Statement on use and care of animals and regulatory compliance

During the study, care and use of animals were conducted in accordance with principles outlined in the current Guide to the Care and Use of Experimental Animals published by the Canadian Council on Animal Care and the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996). LAB Research, Inc.'s facility is AAALAC accredited. Data were obtained under studies conducted in accordance with the Good Laboratory Practice (GLP) regulations of the United States Food and Drug Administration (21 CFR Part 58 and subsequent amendments).

2.2. Animal housing and preparation

The experimental population comprised forty (40) male Sprague–Dawley rats (242–315 g, 59–65 days), eighteen (18) Beagle dogs (6 males and 12 females, 8–18 kg, 1–4 years) and eight (8) cynomolgus (*Macaca fascicularis*) monkeys (4 males and 4 females, 2.5–4.6 kg, 3 to 5 years old). The animal room environment was controlled and monitored continuously (targeted ranges: temperature 21 ± 3 °C, humidity 30–70%, 12 h light, 12 h dark, 10–15 air changes per hour). Rats were fed a standard rodent chow *ad libitum* (Teklad Certified 18% Rodent Diet #2018C). Dogs received a standard certified commercial dog chow (400 g of Harlan Teklad Certified 25% Lab Dog Diet #8727C) over a 24-h feeding period. A standard certified commercial primate chow (Teklad Certified Global 25% Primate Diet #2055C) was made available to each monkey twice daily. Clinical signs were evaluated at cage side at least once daily, and a detailed clinical examination was performed at transfer and once weekly throughout the studies.

2.3. Respiratory monitoring system

Respiratory rate (RR), tidal volume (TV) and minute volume (MV) were recorded continuously at a sampling frequency of 200 Hz in all species. Respiratory function was monitored in rats using head-out plethysmographs (Model PC-H2, SCIREQ, Inc., Montreal, QC, Canada) with opaque headrest connected to a pneumotachometer (Model 8420 with a 5 L/min capacity, Hans Rudolph, MO, USA). The headrests were such that the nose of the rats was open to ambient air but visual and auditory stimulations were attenuated providing a more comfortable environment for the animals. Airflow signals from rat pneumotachometers were monitored using precision differential pressure transducers (Model UT-PDP-02, 0.2 kPa nominal, SCIREQ, Inc., Montreal, QC, Canada) connected to the data acquisition matrix (In expose-08, Data Acquisition Controller 8 channels, SCIREQ, Inc., Montreal, QC, Canada) and a real-time respiratory analyzer (flexiWare 5.3.1, SCIREQ, Inc., Montreal, QC, Canada).

Respiratory monitoring was performed in conscious Beagle dogs as previously described (Authier et al., 2008). In brief, canine respiratory function was monitored using a computerized system composed of a Data Acquisition Controller (DAC 8, Scientific Respiratory Equipment Quebec, Inc. (SCIREQ), Montreal, QC, Canada) using a real-time respiratory analyzer (flexiWare 5.1, SCIREQ, Inc., Montreal, QC, Canada) connected to pneumotachometer (Model 3719 with a 100 L/min capacity, Hans Rudolph, MO, USA) with individual heater controllers (Hans Rudolph, MO, USA) connected to a face mask.

Respiratory function was monitored in conscious cynomolgus monkeys using a computerized system composed of a Data Acquisition Controller (In expose-08, Scientific Respiratory Equipment Quebec, Inc. (SCIREQ), Montreal, QC, Canada) connected to a computer (OptiPlex GX280 Workstation, Dell, Dallas, TX, USA) with a real-time respiratory analyzer (flexiWare 5.1, SCIREQ, Inc., Montreal, QC, Canada). The respiratory hardware included pneumotachometer (Model 3500 with a 35 L/min capacity, Hans Rudolph, MO, USA) with individual heater controller (Hans Rudolph, MO, USA). Monkeys were acclimated to a restraining chair and a transparent plexiglass helmet (Lomir Biomedical, Notre-Dame-de-l'Île-Perrot, QC, Canada) with a bias airflow (inexpose pump module 2.5 lpm, SCIREQ, Inc., Montreal, QC, Canada) and continuous helmet pressure monitoring (Model UT-PDP-25, 2.5 kPa nominal, SCIREQ, Inc., Montreal, QC, Canada). Helmet pressure was maintained neutral to ambient pressure.

Rats, dogs and monkeys were acclimated to respiratory monitoring on three (3) different occasions before initiation of treatment. Rats and dogs that did not tolerate the respiratory monitoring were replaced by animals kept in the same experimental conditions. Respiratory monitoring was well tolerated by all monkeys assigned to the study and no replacement was required. On the day of dosing, rats were placed in the head-out plethysmograph at least 30 min before initiation of baseline monitoring. Dogs and monkeys were placed in the restrainer with the mask or helmet at least 5 min before initiation of baseline monitoring.

2.4. Positive and negative control drugs

Saline (Baxter, ON, Canada), albuterol (Ratiopharm, Inc., QC, Canada), methacholine (Sigma–Aldrich, ON, Canada) and remifentanil (Ultiva®, Abbott Laboratories Ltd., ON, Canada) were administered to conscious rats, dogs and monkeys at doses selected based on historical data obtained in previous studies conducted at LAB Research and adjusted for body surface area (BSA) to induce slight and moderate to severe respiratory effects as presented in Table 1. The dose volume was 2.0 mL/kg for rats, and 0.2 mL/kg for dogs and monkeys (all intravenous agents). Rats were dosed by tail vein injection using a butterfly catheter while a temporary catheter previously placed in the cephalic or saphenous vein was used for dogs and monkeys. All administrations were performed with minimal handling to minimize interferences. Albuterol was administered using a pressurized metered-dose inhaler with a holding chamber and a mask. Monitoring was initiated at least 15 min before dosing in all species. Respiratory data during the first 20 min after administration was used for comparison between species given the rapid onset of pharmacological effects with selected agents.

2.5. Data analysis

Data were averaged every 5 min and results following positive control drugs compared with saline administration. For rats, one

Table 1
Dose levels of positive control drugs.

	Route	Rats	Dogs	Monkeys
Saline	IV	–	–	–
Albuterol (μg/kg)	Inhalation	400	100	100 200
Methacholine (μg/kg)	IV	28 136	2 8	3.4 13.5 68.0
Remifentanil (μg/kg)	IV	14	4	3.4 6.8

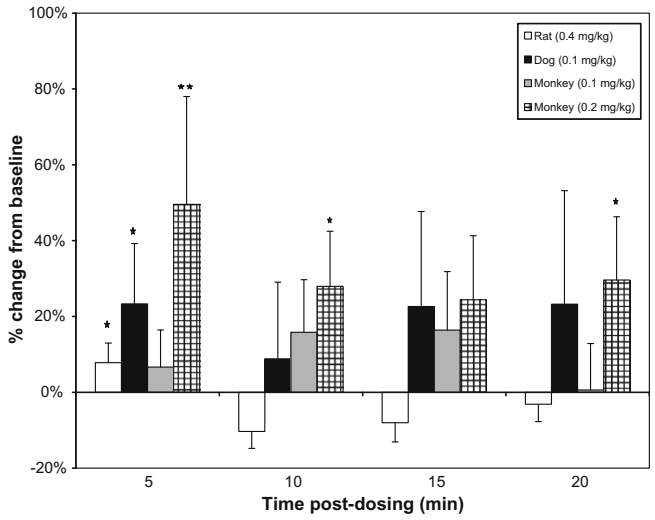

Fig. 1. Tidal volume (TV) after albuterol administered by inhalation to Sprague–Dawley rats ($n = 8$), Beagle dogs ($n = 5$) and cynomolgus monkeys ($n = 8$). Overall group difference when compared with saline was significant for rats ($p < 0.05$), dogs ($p < 0.05$) and monkeys at 200 μg/kg ($p < 0.01$).

(1) min averages at peak effect were also calculated. Normality of distribution was evaluated using the Shapiro–Wilk test. The

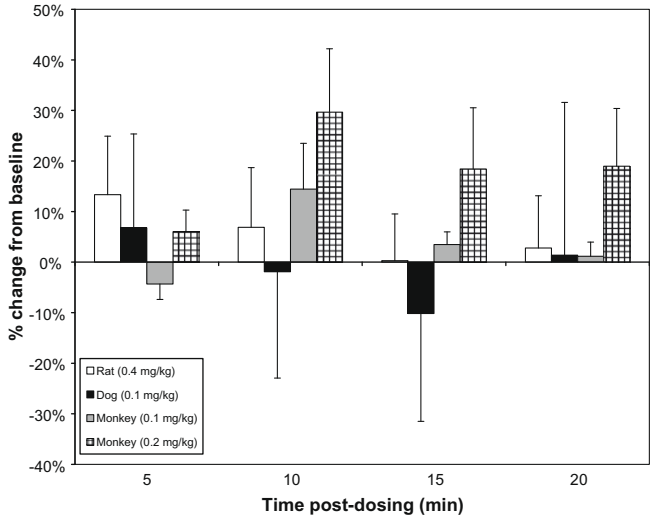

Fig. 2. Respiratory rate (RR) following albuterol administered by inhalation to Sprague–Dawley rats ($n = 8$), Beagle dogs ($n = 5$) and cynomolgus monkeys ($n = 8$). Overall group difference when compared with saline using ANOVA was not significant for any of the three species during the 20 min monitoring period with 5 min averages.

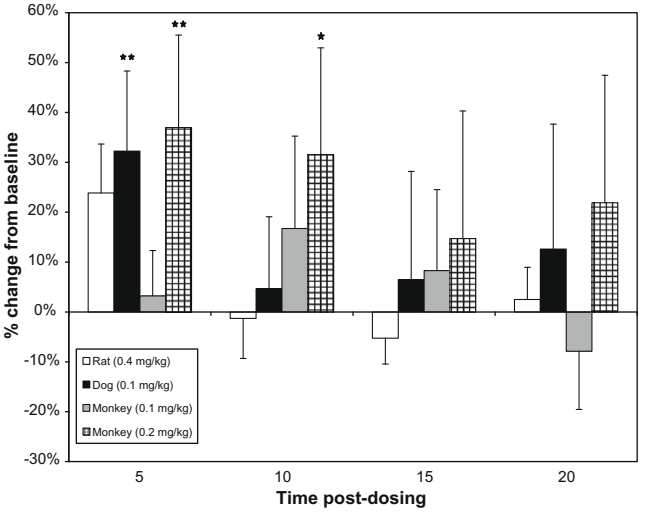

Fig. 3. Minute volume (MV) following albuterol administered by inhalation to Sprague–Dawley rats ($n = 8$), Beagle dogs ($n = 5$) and cynomolgus monkeys ($n = 8$). The overall group difference was statistically significant when compared with saline for dogs ($p < 0.01$) and monkey at 200 μg/kg ($p < 0.01$), while overall group difference did not reach statistical significance in rats when compared with saline. $^{**}p < 0.01$.

Levene test was used to examine the homogeneity of group variances. When both of these tests were found to be non-significant, analysis of variance (ANOVA) was considered appropriate. Whenever the overall group differences were shown significant (*F*-test for ANOVA), then pair-wise comparisons were conducted using Dunnett's test for ANOVA. Comparisons among dose levels at each timepoint were done using a *T*-test and including the Satterthwaite method in presence of heterogenous group variances. Results are presented as mean values ± standard error of the mean (SEM).

3. Results

Albuterol administered by inhalation induced an increase in TV (Fig. 1) in rats, dogs and monkeys when using an analysis with 5 min averages for a 20 min monitoring period compared with sal-

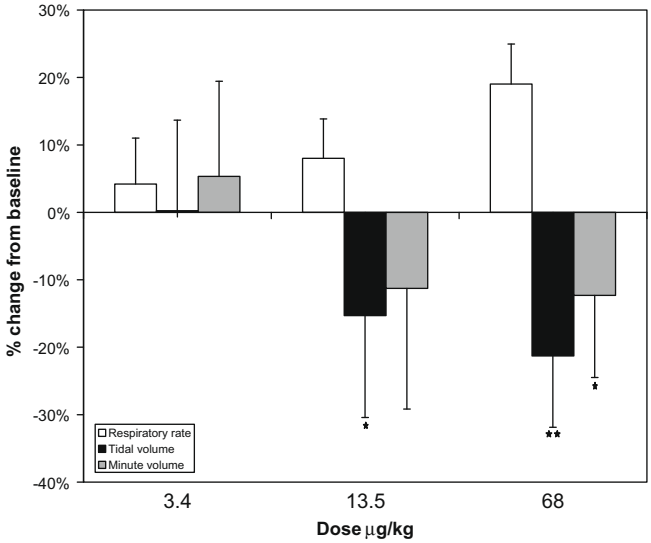

Fig. 4. Respiratory monitoring following methacholine bolus administration (IV) to cynomolgus monkeys ($n = 8$). Overall difference was significant for tidal volume ($p < 0.01$) and minute volume ($p < 0.01$) and significance at each dose is presented above. A trend to compensatory increase in respiratory rate was observed following methacholine administration at 13.5 and 68 μg/kg. $^{*}p < 0.05$, $^{**}p < 0.01$.

ine treatment. Changes to RR were not statistically significant (Fig. 2) in any species during the 20 min monitoring period following treatment, while MV was significantly increased in dogs and monkeys (Fig. 3).

Monkeys presented a decrease in TV ($p < 0.01$) and MV ($p < 0.01$) following administration of methacholine with a trend to a compensatory increase in RR at higher doses (Fig. 4). In contrast, respiratory monitoring in dogs differed with the dose of methacholine: at low dose (2 μg/kg), only MV presented a statistically significant change associated with a decrease in TV and an increase in RR at T10 min; at high dose (8 μg/kg) dogs presented an initial increase in RR ($p < 0.01$) followed by an increase in TV ($p < 0.05$) associated to a decrease in RR and a sustained increase in MV ($p < 0.05$) (Fig. 5). In rats, systemic administration of methacholine resulted in dose-dependent effects: at low dose (28 μg/kg), no statistically significant effect was observed (Fig. 6), but at high dose (136 μg/kg), methacholine induced a significant increase in TV ($p < 0.01$) with a decrease in RR ($p < 0.05$) (Fig. 6).

As expected for a potent μ-agonist opioid, remifentanil induced a significant respiratory depression in all three species (Table 2). Rats presented an initial and transient (at T5) decrease in RR, immediately compensated by a transient (at T10 and T15) increase in TV, without significant change in MV. When using one (1) min averages starting at pharmacological onset, RR (−37.4% ± 4.2%) and MV (−32.6% ± 5.3%) were significantly decreased ($p < 0.01$) in rats following 14 μg/kg remifentanil administration. Dogs presented a biphasic response with hypoventilation followed by a delayed phase of hyperventilation with increased RR. Panting was noted in some dogs when adverse respiratory effects were seen which complicated respiratory analysis with this species. In monkeys, apnea was observed in three out of seven monkeys at high dose (6.4 μg/kg), which prompted reversal of the opioid effects with naloxone (400 μg/mL, IV).

4. Discussion

Species differences are recognized by regulators and dictate selection of maximum safe starting dose during initial clinical trials in healthy volunteers (U.S. Food and Drug Administration,

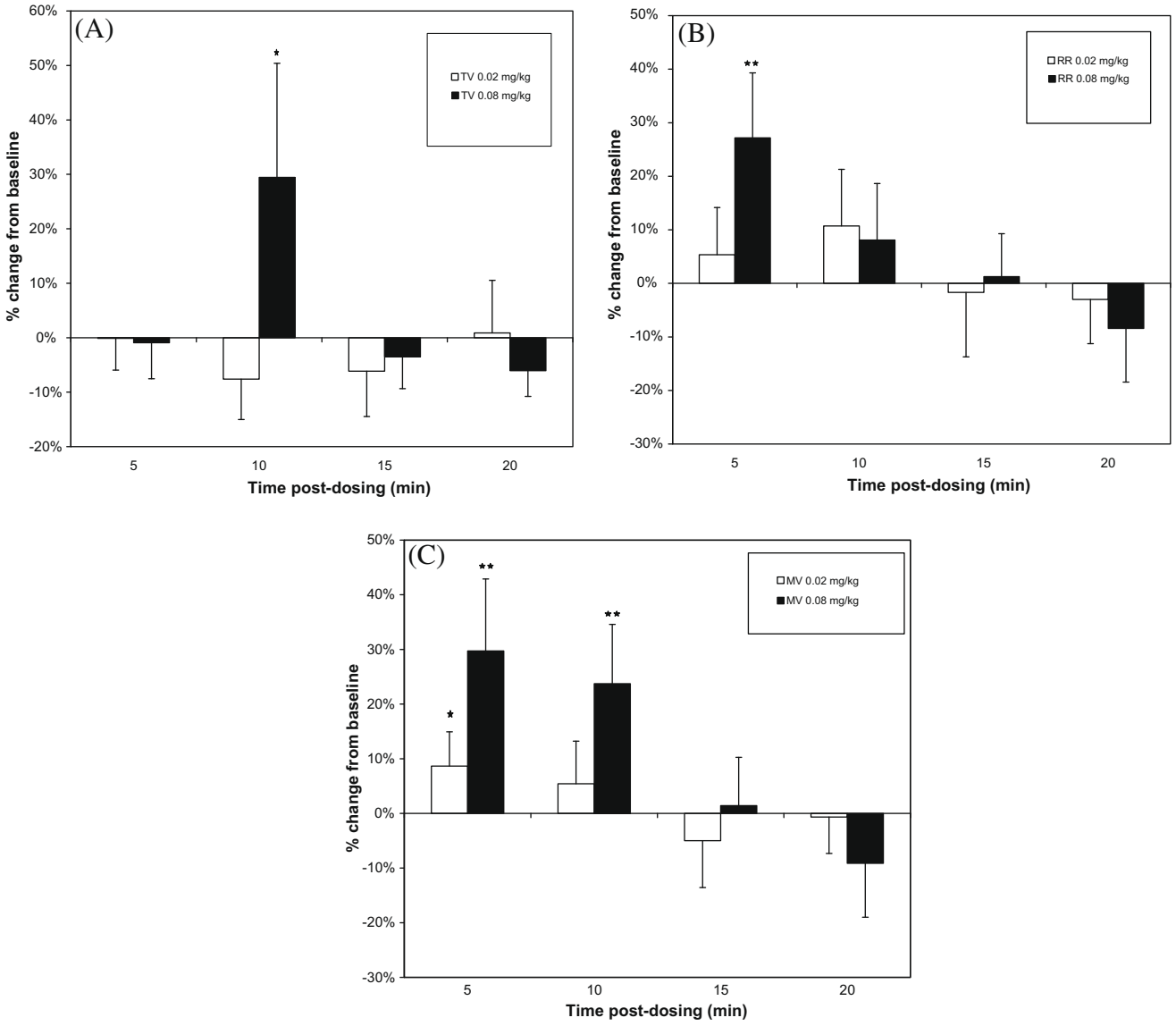

Fig. 5. Tidal volume (A), respiratory rate (B) and minute volume (C) following methacholine bolus administration (IV) to Beagle dogs ($n = 8$). The overall difference was significant for MV at 2 μg/kg ($p < 0.01$) and for all three parameters at 8 μg/kg (RR, $p < 0.01$; TV, $p < 0.05$; MV, $p < 0.05$). At 8 μg/kg, a sustained increase in MV is explained by a biphasic response, with an initial increase in RR, followed by an increase in TV while RR is returned toward baseline. * $p < 0.05$; ** $p < 0.01$.

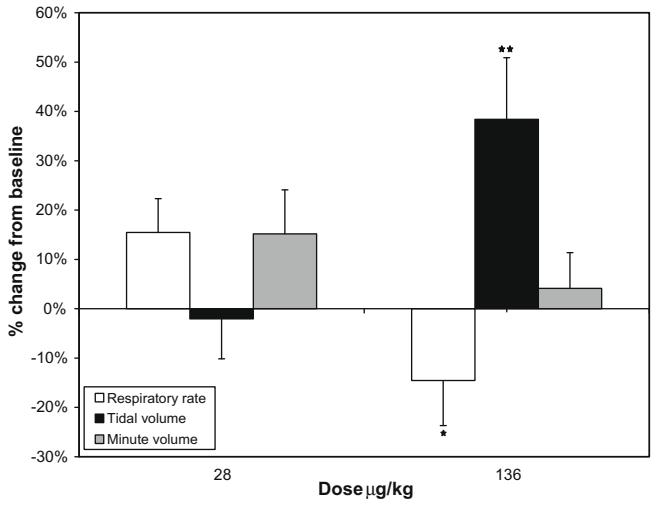

Fig. 6. Respiratory monitoring (5 min average at pharmacological onset) following methacholine bolus administration (IV) to Sprague–Dawley rats ($n = 8$). When compared with saline, RR was decreased ($p < 0.05$) and TV was increased ($p < 0.01$) after methacholine at 136 µg/kg. The changes in RR ($p = 0.07$) and MV ($p = 0.11$) at 28 µg/kg did not reach statistical significance. $^{*}p < 0.05$, $^{**}p < 0.01$.

2005). Allometric correlations have been reported between species for metabolic rates but also for physiological parameters such as heart rate (West et al., 2002), cardiac output, respiratory rate and ventilation (Lindstedt and Schaeffer, 2002). Allometric correlation between body weight and MV has been recognized for several decades (Guyton, 1947; Stahl, 1967). Inter-species correlations are recognized *de facto* in toxicology which uses models from multiples species to predict the human response. Allometric scaling of respiratory parameters is also a central exercise in inhalation toxicology studies for dose estimation. A formula ($V(m) = 0.608\ BW^{0.852}$) to estimate respiratory MV adapted to laboratory animals (mouse, rat, dog and monkey) was recently published by the Association of Inhalation Toxicologists (AIT) (Alexander et al., 2008). When using the recent AIT formula, the predicted MV in rats (191 ± 3 mL/min) and monkeys (1759 ± 100 mL/min) were closer to actual respiratory measurements following saline administration than the predicted MV calculated (rats 166 ± 2 mL/min; monkeys 1368 ± 74 mL/min) with the Bide formula ($V(m) = 0.499\ BW^{0.809}$) which is commonly used for dose calculation in inhalation toxicology studies (Bide et al., 2000). In contrast, the Bide's formula (3846 ± 196 mL/min) was superior to the recent AIT formula (5227 ± 225 mL/min) for calculated MV in dogs. The importance to evaluate standard formulas with in-house data was demonstrated for QT correction formulas (Tattersall et al., 1998). Similarly, allometric formulas used in dose calculation for inhalation toxicology studies benefit from qualification using actual MV values recorded in experimental conditions that prevail in each laboratory.

In humans, systemic administration of albuterol induces a significant increase in TV and MV (Sorbini et al., 1984) which is thought to result from an increased metabolic rate and serum lactate (Tobin et al., 2006). Similar to humans, monkeys and dogs presented a significant increase in TV and MV, while only increase in TV reached statistical significance in rats when compared with Saline. The duration of effects was longer in monkeys when compared to dogs at equivalent doses (100 µg/kg in dogs and 200 µg/kg in monkeys). This could be related to a greater respiratory reserve in dogs which resulted in a rapid compensation. Albuterol by inhalation induces tachycardia with a decrease in systolic pressure in Beagle dogs (Petruska et al., 1997). Similarly, rats presented tachycardia following inhaled albuterol at doses of 84 µg/kg (4.8 times lower than the dose used in the current study). Doses up to 7.5 times higher than the high dose (200 µg/kg) used in this study did not produce significant cardiovascular effects in cynomolgus monkeys. Cardiovascular effects of albuterol, also reported in healthy volunteers (Corea et al., 1984), may contribute to respiratory changes when using this positive control drug in respiratory safety models and in humans. Differences in ventilatory responses observed between the three models may be due to species specific sensitivity to cardiovascular and/or respiratory effects. Cardiopulmonary dependency is an emerging concept in safety pharmacology that often requires monitoring of the respiratory and cardiovascular systems simultaneously in the same animals to identify correlation between the two systems.

Methacholine at the high dose (8 µg/kg in dogs and 13.5 µg/kg in monkeys) induced diametrically opposite effects with a significant increase of MV in dogs while monkeys presented a decreased MV. Whereas TV was consistently decreased in monkeys at high dose methacholine, in dogs, TV was initially slightly reduced as previously reported (Savoy et al., 1982) and associated with an abrupt increase in RR, followed by a second phase of respiratory response including increase in TV and return toward baseline values for RR. Such results suggest that the dog is highly responsive to the bronchoconstrictive effects of methacholine resulting in an abrupt increase in RR. Healthy humans are reported to present a decreased MV and TV (Fujimori et al., 1996) similar to the response observed in monkeys. Similar to dogs, the rats presented a response different from humans and monkeys with an increase in TV and a decrease in RR at the doses used in the current study. It remains that all three (3) species presented significant ventilatory changes following bronchoconstriction with methacholine and are considered suitable models to detect the presence of respiratory liability. Core battery safety pharmacology studies should identify potential adverse effects in human patients. The response to bron-

Table 2
Respiratory parameters following remifentanil (5 min averages starting at injection).

Control articles		Dose (µg/kg)	Respiratory rate (b/min)		Tidal volume (mL)		Minute volume (mL/min)	
			Pre-Rx	Post-Rx	Pre-Rx	Post-Rx	Pre-Rx	Post-Rx
Sprague–Dawley rats	($n = 8$)	0	200.2 ± 9.7	204.0 ± 12.6	1.42 ± 0.20	1.54 ± 0.07	264.5 ± 10.2	315.6 ± 37.2
	($n = 8$)	14	188.7 ± 10.4	164.4 ± 7.3*	1.52 ± 0.07	1.68 ± 0.09*	271.6 ± 13.5	262.4 ± 12.0
Beagle dogs		0	17.1 ± 4.5	16.4 ± 3.3	208 ± 59	217 ± 75	3409 ± 709	3443 ± 1201
	($n = 7$)	4	15.8 ± 2.3	11.6 ± 0.8*	270.7 ± 19.4	243.2 ± 24.3	3913 ± 440	2339 ± 193**
Cynomolgus monkeys		0	51.8 ± 2.1	50.5 ± 3.7	41.1 ± 4.1	41.1 ± 3.5	2088 ± 170	2154 ± 229
	($n = 8$)	3.4	49.2 ± 3.2	39.6 ± 2.5	39.4 ± 3.5	23.0 ± 3.2**	1864 ± 163	911 ± 165**
	($n = 7$)[a]	6.8	50.4 ± 4.4	41.7 ± 3.4	41.6 ± 3.9	28.4 ± 4.5*	1945 ± 143	1297 ± 287*

* $p < 0.05$.
** $p < 0.01$.
[a] Effects of remifentanil was reversed with naloxone IV for three out seven animals due to severe apnea.

choconstriction, although different between species, will yield the same conclusion; identification of a potential respiratory (adverse) effect in humans which will trigger appropriate monitoring during early clinical trials.

Remifentanil, a potent μ-agonist opioid, leads to respiratory depression in humans (Smith et al., 1997), which translates into decreased MV. Expected effects were observed in all species, but in rats the depression was more evident with the analysis at one (1) min interval. Indeed for rats, analysis at onset of pharmacological effects with one (1) min averages revealed higher sensitivity to detect changes after remifentanil administration. The difference in statistical results between one (1) and five (5) min averages highlights the importance of post-acquisition data analysis which needs to be tailored to each test article in order to capture pharmacological effects. On the other hand, adapting statistical analysis *at posteriori* to capture an unknown pharmacodynamic response is a challenging issue in regulatory toxicology where any modification to the analysis plan would raise concerns on potential bias.

Results from the current study suggest that the ventilatory response to bronchoconstriction in the monkey is closer to humans. These pharmacodynamic similarities correlate with anatomic, physiologic and histologic characteristics of monkeys that resemble humans while dogs and rats present lower level of homology with humans. Monkeys (Dungworth et al., 1975), dogs (Takenaka et al., 1998) and humans (Saetta et al., 1994) have several generations of respiratory bronchioles while rats have either no respiratory bronchioles of a single generation (Tyler and Julian, 1991; Saetta et al., 1994). The number of alveolar pores, which facilitates collateral ventilation, is similar in dog, monkey and human alveolus but lower in rats (Port et al., 1977). Submucosal glands are observed throughout the bronchial tree of dog (Takenaka et al., 1996), monkeys (El-Bermani and Grant, 1975) and humans (Scott, 1973) while rats do not have bronchial glands (Jeffery, 1983). Respiratory neural organization in monkeys, dogs and rats is comparable to humans (Kastner and Gauthier, 2008) with respiratory centers (inspiratory, expiratory, pneumotaxic and apneustic) located in the medulla oblongata and multiple nervous effectors controlling ventilation such as the phrenic and intercostal nerves (King, 2005). Despite central similarities the pulmonary innervation presents differences between species. The rat lung innervation presents significant differences when compared with monkeys (El-Bermani, 1978) while lung innervation in dogs is comparable to monkeys (Knight et al., 1981). Will anatomical and physiological similarities between monkeys and humans translate into increased predictive value with this species? If so, will increased predictive value alter the decision making process during drug development? The answer resides in an integrated risk assessment of the toxicology testing plan. Despite differences between humans and rats, the rat model is widely accepted in pharmaceutical research (Tschernig et al., 2008) and this species remains predictive of the patient response. Clinically significant alterations to respiratory parameters were noted in rats with all positive control drugs used in this study supporting the use of the three species for respiratory safety investigations.

The current study holds some limitations and complete dose–response curves would be needed to compare species sensitivity. It remains that dose levels that were used reliably induce bronchoconstriction, bronchodilation and respiratory depression given the well-characterized positive control drugs that were selected. As observed in the current study, dogs occasionally present panting when stressed or in response to drug-induced adverse effects. Panting during respiratory monitoring acclimation in dogs triggers exclusion of individuals. When present, panting decreases accuracy of the ventilatory measures and increases artefacts due to excessively rapid ventilation of the respiratory dead space. Occasional panting is an inconvenient of the canine model and the rat can be preferred for respiratory safety testing when these two species are selected for toxicology studies. In contrast, the cynomolgus monkey maintains a tidal breathing pattern and the inclusion of non-invasive respiratory (ventilatory) investigations in toxicology studies may present some advantages over ventilatory assessments in rats.

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