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Original article

Respiratory mechanics: Comparison of Beagle dogs, Göttingen minipigs and Cynomolgus monkeys ☆

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ABSTRACT

Introduction: When the no observed adverse effect level (NOAEL) is determined by respiratory safety pharmacology, follow-up studies are warranted and may include airway resistance and compliance. Respiratory mechanics in commonly used large animal species (Beagle dogs, Cynomolgus monkeys, and Göttingen minipigs) were compared. **Methods:** Eighteen animals were used (3/sex/species) in an anesthetized model (propofol infusion) with pancuronium as a neuromuscular blocker. Parameters of respiratory mechanics were evaluated at baseline and at peak drug effect. Resistance (R_{rs}) and elastance (E_{rs}) were measured by applying a single frequency forced oscillation (0.5 Hz) to the subject's airway opening and fitting the flow, volume and pressure data to the single compartment model of the lung. Increasing doses of intravenous (IV) methacholine were administered in all three species, as well as doubling aerosolized concentrations of the same bronchoconstrictor agent before and after inhaled albuterol. **Results:** The slope of the IV methacholine dose-response curve for R_{rs} was similar in dogs and monkeys and both species differed from minipigs, which showed greater reactivity. At the highest IV dose tested, minipigs also reached higher levels of bronchoconstriction than the other two species. They were followed, in decreasing order, by dogs and monkeys. Albuterol induced a significant decrease in the slope of the dose-response curve only in dogs and monkeys. **Discussion:** Scientific literature is available on respiratory mechanics in monkeys and dogs but not in minipigs. Our results suggest that minipigs were more reactive than dogs and monkeys to IV methacholine while less sensitive to inhaled albuterol.

1. Introduction

The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) guideline about Safety Pharmacology Studies for Human Pharmaceuticals (S7A) (U. S. Food and Drug Administration, 2001) was adopted in November 2000 and has since served as a steering document to define the scope of safety pharmacology studies. Although occasional criticism was expressed on both sides, the S7A guideline was generally well accepted by the drug development industry and the regulators. As a result of S7A implementation, homogeneity of investigational new drug (IND)-enabling studies has increased. Most respiratory safety pharmacology studies are conducted using a conscious rat model (Lindgren et al., 2008) in which parameters such as respiratory rate (RR), tidal volume (TV), and minute volume (MV) are monitored. On the other hand, the last decade has seen a rise in the number of agents in development where the use of rats is not relevant (*e.g.* target not present in rats). In such cases, respiratory safety pharmacology will be conducted in a large animal species such as monkeys or dogs.

In conscious subjects, drug-induced ventilatory and oxygenation changes can not only originate from direct pharmacological effects on the respiratory system (*e.g.* increase in airway resistance), but also from secondary effects on the central nervous system (*e.g.* sedation), cardiovascular system (*e.g.* hypotension) or other consequences of toxicity. Therefore, common ventilatory parameters (*i.e.*, RR, TV and MV) are not sufficient to determine the underlying mechanisms of respiratory changes (pulmonary, central nervous system or cardiovascular).

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The "no observed adverse effect level" (NOAEL) is defined as the dose level at which all observed effects are considered as being "non-adverse". The NOAEL dictates the maximum recommended starting dose in phase 1 clinical trial and is determined based on safety pharmacology and/or toxicity studies as defined in the Food and Drug Administration guidance to estimate a safe starting dose (U. S. Food and Drug Administration, 2005). When the NOAEL is based on the respiratory safety pharmacology study, follow-up studies are warranted and may include airway resistance and compliance or elastance (U. S. Food and Drug Administration, 2001). The current project aimed to compare models of respiratory mechanics in monkeys and dogs which are large animal species commonly used in safety pharmacology studies using positive control drugs. In addition, we included the Göttingen minipig as another comparative species, which has increased in popularity and for which there is a paucity of historical data for functional respiratory assessments. Another specific objective of the study was to quantify in these three different models the maximal response at a given dose, as well as the sensitivity (leftward or rightward shift) and the reactivity (rate of change) to a challenge using drugs recognised as being respectively bronchoconstrictor (methacholine) and bronchodilator (albuterol).

2. Materials and methods

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

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 Institute of Laboratory Animal Resources. CiToxLAB – North America's facility is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), and the procedures were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) prior to conduct. All procedures were conducted as per Standard Operating Procedures (SOPs) in place.

2.2. Animal housing and preparation

The experimental population comprised six (6) Beagle dogs (*Canis familiaris*) (3 males and 3 females, 9.3–11.4 kg, 8 to 14 months), six (6) Cynomolgus (*Macaca fascicularis*) monkeys (3 males and 3 females, 2.7–4.0 kg, 3–4 years) and six (6) Göttingen (*Sus scrofa*) minipigs (3 males and 3 females, 12.4–14.2 kg, 5–6 months). The animal room environment was controlled and monitored continuously (targeted ranges: temperature 18–24 °C, humidity 30–70%, 12 h light, 12 h dark, 10–15 air changes per hour). Dogs received a standard certified commercial dog chow (400 g of Certified 25% Lab Dog Diet 8727C, Harlan Teklad, Madison, WI, USA) over a 24-h feeding period. A standard certified commercial swine chow (Certified Miniswine Diet 7037CTM, Harlan Teklad) was available to each minipig twice daily. A standard certified commercial primate chow (Certified Global 25% Primate Diet 2055C, Harlan Teklad) 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. Animal preparation and monitoring

Anaesthesia was induced with propofol (PropoFlo™, Abbott Laboratories Ltd., Montreal, QC, Canada) given intravenously (IV) into the cephalic vein to effect (6 mg/kg, 10 mg/mL) followed by continuous infusion (24 mg/kg/h to 42 mg/kg/h, as needed). Animals were carefully oro-tracheally intubated. Lidocaine spray (10% w/w) was administered onto the glottis prior to intubation, when necessary. An ophthalmic ointment was applied to both eyes to prevent drying of the cornea. The animal was then connected to a *flexiVent* system (SCIREQ Inc., Montreal, Canada) and ventilated with 21% oxygen (O_2) using a tidal volume sufficient to maintain a peak inspiratory pressure of 18–20 cmH_2O, a respiratory rate of 15–25 breaths/min and a ratio I:E of 66.67%. To minimize respiratory artefacts that would interfere with the forced oscillation measurement signal, pancuronium (Pavulon™, Merck Canada, Inc., Kirkland, QC, Canada), a neuromuscular blocker, was administered IV (0.05 to 0.11 mg/kg). Continuous monitoring was performed using a pulse oximeter (Surgivet, Smiths Medical PM, Inc., Waukesha, WI, USA) and a capnometer (Ohmeda Model 5052, Louisville, CO, USA). More specifically, it included pulsatile O_2 saturation of haemoglobin (SpO_2), inspiratory and end-tidal carbon dioxide (CO_2) levels, heart rate and RR. Specific respiratory monitoring included RR, TV and MV for all animals. Lactated Ringer's solution was also administered IV throughout the procedure at a rate of 10 mL/kg/h. In addition, arterial blood samples were taken 5 min after dosing, in all dogs. The femoral artery was used as a collection site using an indwelling catheter. Parameters evaluated were pH, CO_2 and O_2 partial pressure ($PaCO_2$, PaO_2 respectively), bicarbonates (HCO_3^-), total CO_2 (TCO_2), base excess in extracellular fluids (BEecf) and arterial O_2 saturation of haemoglobin (SaO_2), as well as electrolytes (Na^+, K^+, and ionized Ca^{2+}), packed cell volume (PCV), haemoglobin and glucose concentrations (i-STAT®, Heska, Flamborough, ON, Canada). At the end of the experiment, neostigmine (Prostigmin™, Merck Canada, Inc.) was injected subcutaneously (0.005 mg/kg) to reverse the effects of the neuromuscular blocker at completion of monitoring.

2.4. Respiratory system mechanics measurements.

The mechanical properties of the respiratory system were assessed using the forced oscillation technique (McGovern et al., 2013). More specifically, respiratory mechanics was assessed at baseline and following challenge with bronchoactive drugs using a low amplitude (10 mL/kg), single frequency (0.5 Hz) forced oscillation signal imposed at the subject's airway opening during a brief apneic period (2 s). Following bronchoconstrictor challenge, measurements were taken in an automated manner every 30 s for a period of 2–3 min in order to capture peak response or to see a doubling of the baseline resistance value. The *flexiVent* 5.2 ® software (SCIREQ, Montreal, Canada) calculated dynamic respiratory system resistance (R_{rs}) and elastance (E_{rs}) using the linear first-order single compartment model in anaesthetised animals. Capturing airway opposition to an airflow signal, R_{rs}, is useful as a measure of the comparative resistance of the respiratory system, which includes the resistance of central and peripheral airways as well as lung tissues (Shalaby et al., 2010). As inverse of compliance, E_{rs} represents elastic rigidity of the respiratory system and resistance to deformation. Each dataset was associated with a coefficient of determination (COD), which is a quality control parameter evaluating the data fit to the mathematical model used in the analysis. It was used as an exclusion factor for values of respiratory mechanics in unconscious animals. All datasets associated with COD values below 0.9 were excluded from the analysis. Subsequently, a trained veterinarian (SA) reviewed all respiratory mechanics values with a COD above 0.9, before considering their inclusion in the analysis.

2.5. Experimental protocol

At the start of each experiment, lung recruitments manoeuvres were performed in order to recruit all closed lung areas and to standardize lung volume history. This was done by inflating the subject's lungs to 30 cmH_2O over a 3 s period and held at that pressure for 3 s using a pressure-driven perturbation. After determination of baseline respiratory mechanics values, methacholine (Sigma-Aldrich, Oakville, ON, Canada) was administered IV in the cephalic or saphenous vein to anaesthetised dogs, pigs and monkeys at increasing dose (3.4 [1x], 13.5 [4x], and 68.0 [20x] μg/kg) (Fig. 1). Dosages were selected based on historical data obtained in previous studies conducted at CiTox-

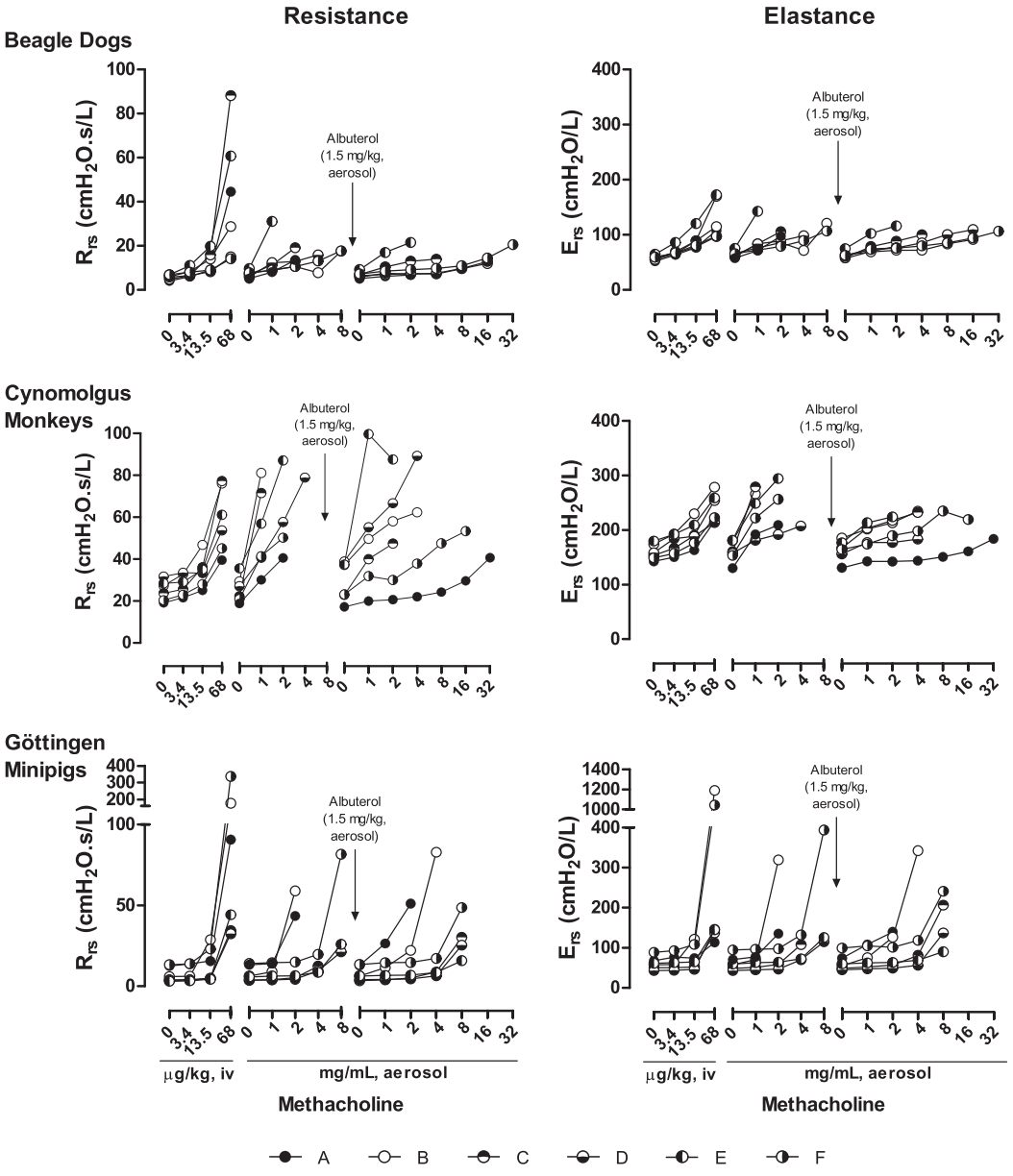

Fig. 1. Individual resistance and elastance values in all three species at baseline and following bronchoconstrictor and bronchodilator challenges. A, B, *etc.* indicate individual animals.

LAB – North-America. Lung recruitments manoeuvres were performed between dosesas well as after the final dose, where they were repeated until the R_{rs} returned to baseline. In cases where the animal's lung capacity exceeded the piston stroke volume of the ventilator module, the lungs were manually inflated to 30 cmH_2O by clamping the expiratory line over 2–3 breaths. Subsequently, doubling concentrations (1 to 32 mg/mL) of methacholine were given by inhalation using an in-line computer-controlled Aeroneb Lab nebulizer (Aerogen Ltd, Ireland; fine mist model, synchronized with inspiration) until baseline R_{rs} was doubled. Albuterol (Sigma Aldrich, St-Louis, MO, USA) was then given by inhalation by nebulizing to dryness a 1.5 mg/kg dose over a period of 5–6 min, using a similar computer-controlled nebulizer (Aeroneb Lab, standard mist model) and a stock solution at 30 mg/mL (Petruska et al., 1997; Van Scott et al., 2004). The dose was reported to represent a 100 fold multiple the maximal daily clinical dose of Albuterol (Petruska et al., 1997). Lung recruitment manoeuvres were then performed until return to baseline, and bronchoprotection was assessed by re-challenging the subject's lungs with doubling concentrations (1 to 32 mg/mL) of inhaled methacholine until baseline R_{rs} had once again doubled. The change in the methacholine concentration-response curve slope relative to pre-albuterol as well as the provocative concentration required to induce a 200% increase in resistance (PC_{200}) after inhalation of methacholine were calculated for each parameter and compared between species. The slopes of individual dose response curves (e.g. after iv methacholine as well as before and after aerosolized methacholine) were calculated by linear regression analysis, as previously described in the literature (Walker et al., 2012). More specifically, peak responses (raw data) at each challenge were expressed as their natural logarithm and plotted against the concentration of methacholine. A linear regression analysis was then used to determine the best-fit straight line passing through all the experimental points and to obtain its slope. Similarly, individual PC_{200} values were calculated by fitting a second order polynomial to the raw R_{rs} concentration-response curve and interpolating the curve at the doubling of baseline.

2.6. Data analysis

Results are presented as mean ± standard deviation. Dose-response curves were compared by analysis of variance (ANOVA; GraphPad Prism version5; GraphPad Software, San Diego, USA) for a single factor

Table 1
Baseline values.

Species	Resistance ($cmH_2O.s/L$)	Elastance (cmH_2O/L)	n
Beagle dogs	5.79 ± 1.12	57.76 ± 4.36	6
Cynomolgus monkeys	25.16 ± 4.87*	159.60 ± 14.55*	6
Göttingen minipigs	7.12 ± 4.62	61.46 ± 16.00	6

*Adjusted p value < 0.05 for between-groups comparison.

using the slope of the natural logarithm-transformed response. This was followed by Tukey's multiple comparison test for mean comparison. The peak values recorded after challenge to the different positive control drugs were used to test the maximal response. *A priori* contrasts were done to compare means. The α-threshold was adjusted with the Bonferroni sequential method. To test the sensitivity to albuterol, the slopes of the natural logarithm-transformed response curves before and after albuterol administration were compared using a paired Student's *t*-test. The albuterol-induced change in the slope of the methacholine response curves for R_{rs} and E_{rs} was also calculated and compared across species using a single factor ANOVA. Differences at $p < 0.05$ two-sided were considered statistically significant.

3. Results

Individual subject R_{rs} and E_{rs} values for all three species at baseline and following bronchoconstrictor and bronchodilator challenges are presented in Fig. 1. Average baseline values for each species were calculated and can be found in Table 1. Results showed that baseline R_{rs} and E_{rs} were not different between minipigs and dogs, but were different between monkeys and the other two species, with monkeys having higher R_{rs} and E_{rs} values compared to the other two species.

After IV methacholine, the linearized R_{rs} and E_{rs} dose-response curves were remarkably parallel between species, except for minipigs (Fig. 2A, B). Indeed, a comparison of dose-response curve slopes across species showed that dogs and monkeys had comparable reactivity to IV methacholine for R_{rs} and E_{rs}. Minipigs, on the other hand, reacted more than dogs and/or monkeys, as observable by a statistically greater slope in R_{rs} relative to the two other species (Fig. 2A) and in E_{rs} relative to monkeys (Fig. 2B). In addition, at the highest dosage (68 μg/kg), minipigs presented the highest degree of bronchoconstriction of all three species followed, in decreasing order, by dogs and monkeys (Fig. 2C, D). Average peak values for R_{rs}, and E_{rs} as percentage of variation from baseline after IV injection of methacholine are shown in Fig. 3. *A priori* contrasts reported a significant difference between monkeys and minipigs for R_{rs}, but were not significant for all other comparisons. However, variations from baseline for R_{rs} and E_{rs} were higher in minipigs and lower in monkeys compared to both other species. In terms of variations of R_{rs} from baseline (Fig. 3), minipigs bronchoconstricted approximately 10 times more than monkeys (1460 ± 953% vs 135 ± 56%, respectively; $p < 0.05$) and 2 times more than dogs (1460 ± 953% vs 684 ± 607%, respectively) at the highest methacholine dosage.

Fig. 4 shows individual slopes of inhaled methacholine concentration-response curves pre- and post-albuterol administration for both R_{rs} and E_{rs}. Pre-albuterol, slopes for R_{rs} and E_{rs} were similar between species. Post-albuterol, minipig was the only species to lack a significant decrease in slope for both R_{rs} and E_{rs} (Fig. 4A, B). Consistently, variations in slope from pre-albuterol for R_{rs} and E_{rs} were significantly lower in minipigs in comparison to dogs and monkeys, which had a comparable level of change (Fig. 4C, D). This was associated with a significant increase in the PC_{200} value for resistance in dogs (Table 2). The large variability associated with the post-albuterol PC_{200} in monkeys prevented the analysis from reaching the statistically significant level of $p < 0.05$. There was also no statistical difference between pre- and post-

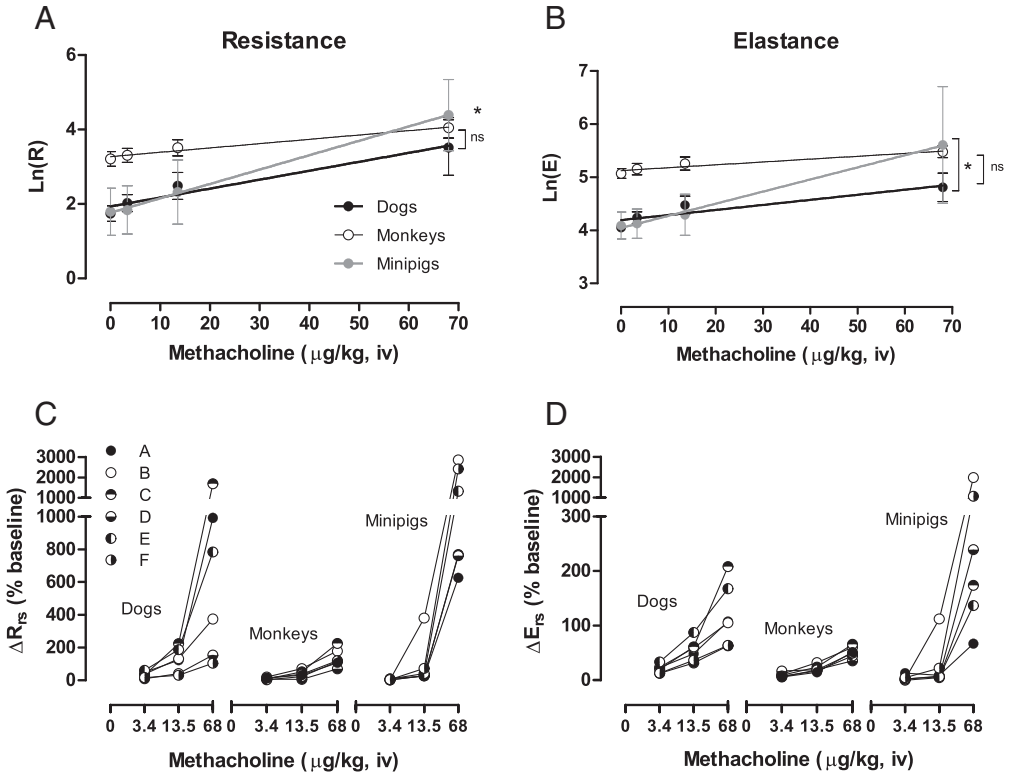

Fig. 2. Linearized resistance (A) and elastance (B) dose-response curves after IV injection of methacholine as well as individual percentages of variation from baseline in all three species (C, D). *Adjusted p value < 0.05 for between-groups slope comparison (ANOVA, n = 6/group).

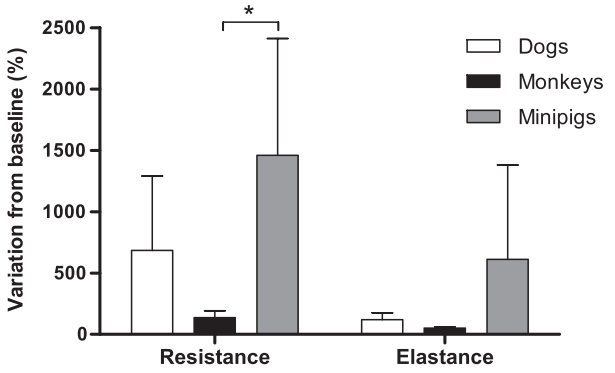

Fig. 3. Variation (maximal response) from baseline for resistance and elastance for each species after exposure to a 68 μg/kg dose of methacholine administered intravenously. *Adjusted p value < 0.05 for between-groups comparison.

albuterol PC_{200} values for resistance in minipigs (Table 2). No changes in arterial blood gases were noted with the ventilatory alterations induced by these positive control drugs.

4. Discussion

The primary goal of the study was to compare respiratory mechanics in three large animal species used in safety pharmacology, Beagle dogs, Cynomolgus monkeys and Göttingen minipigs, at baseline and following challenge with benchmarked positive control drugs. The specific objectives were 1) to quantify the dose-response curves to IV methacholine in order to compare these three species in terms of reactivity and the degree of maximal response; 2) to assess species specific reactivity and sensitivity changes to a broncho-constrictive/-dilative challenge using inhaled methacholine and albuterol, respectively. In our study, the maximal response was analysed by the peak values at the highest IV dosage, the reactivity by the slope of the dose (or concentration)-response curves and the sensitivity by assessing the provocative concentration required to cause a doubling in R_{rs}. However, it is understood that these variables are interlinked, being all derived from the dose-response curve.

Beagle dogs and Cynomolgus monkeys were compared in a previous study by the same group (Authier et al., 2009). Both species presented similar increase in TV and MV in response to a 0.1 mg/kg (15 times lower than in the current study) inhaled dose of albuterol. In monkeys, methacholine IV injection (same three doses as the current study) induced a dose-dependent decrease in TV and MV. However, in dogs, the results were more variable with two doses tested, *i.e.* 2 and 8 μg/kg; the highest dose tested even leading to an increase in TV and MV. When testing the same IV doses (3.4, 13.5 and 68 μg/kg) of methacholine in minipigs (Authier et al., 2011), only the highest dose induced a transient but increase in RR, TV and MV. The current study extended our investigations of potential difference in response to methacholine using a more sophisticated methodology for respiratory mechanics monitoring.

Methacholine can alter pulmonary function by actions on both the airways and the pulmonary parenchyma (Sly & Lanteri, 1991). Methacholine was reported as a positive control drug in dogs (Authier et al., 2009; Sly et al., 1998), minipigs (Authier et al., 2011; Kleinsasser et al., 2007) and monkeys (Authier et al., 2009; Dybas et al., 2006; Madwed & Jackson, 1997). None of those studies measured respiratory system mechanics parameters in more than one species. Methacholine is reported to induce bronchoconstriction, as measured by an increase in R_{rs} and/or E_{rs}, and a decrease in compliance.

In the current study, values of R_{rs} and E_{rs} were higher in monkeys compared to the other two species before drug administration. After methacholine injection or inhalation R_{rs} and E_{rs} increased, as expected. Interestingly, the dose-response curves to IV methacholine were parallel between species, except for minipigs which presented a higher reactivity at the dose range studied. At the highest IV dosage, minipigs had a higher degree of response and monkey a lower degree, compared to the other species (see Fig. 3 illustrating the degree of response for R_{rs}).

Following an aerosolized methacholine challenge, the concentration-response curve slopes before albuterol inhalation (see Fig. 4A and B

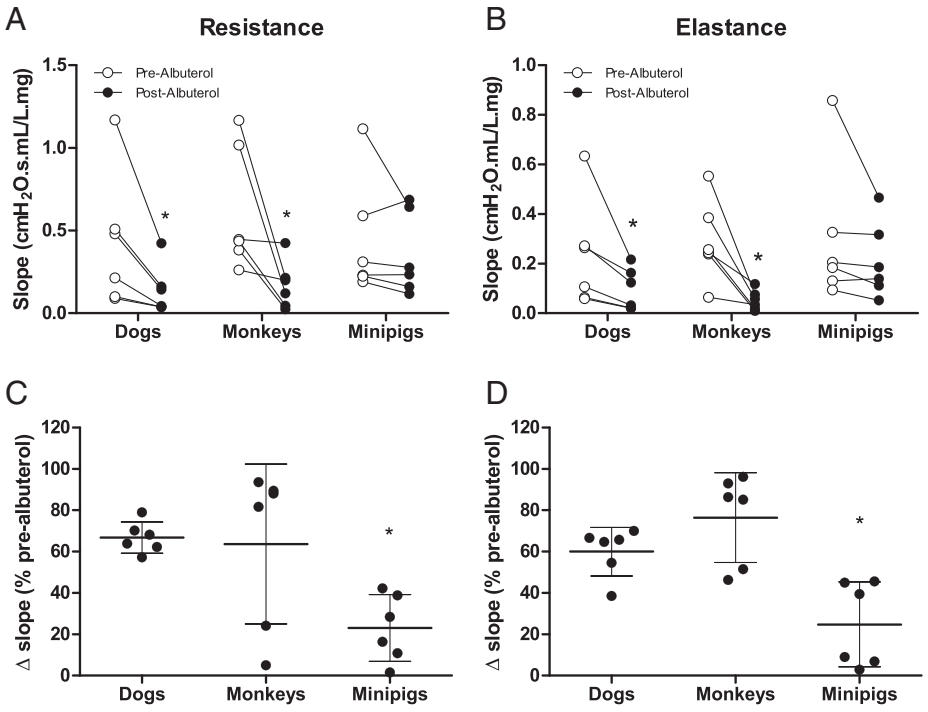

Fig. 4. Individual slopes of inhaled methacholine concentration-response curves pre- and post-albuterol administration for resistance (A) and elastance (B) as well as individual percentages of variation in slope from pre-albuterol in all three species for resistance (C) and elastance (D). *Adjusted p value < 0.05 for between-groups comparison.

Table 2
Mean PC_{200} values for resistance pre- and post-albuterol.

Species	PC_{200} pre-albuterol (mg/mL)	PC_{200} post-albuterol (mg/mL)	p value
Beagle dogs	3.13 ± 2.50	7.33 ± 6.01	0.04
Cynomolgus monkeys	1.38 ± 0.73	6.47 ± 8.26	0.09
Göttingen minipigs	3.08 ± 1.54	3.68 ± 2.27	0.06

illustrating the curve slopes for R_{rs} and E_{rs}) were similar between species. The pattern difference relative to the IV mode of administration could be related to the dose range studied, the distribution of the drug, or to difference in the respiratory system response (Wagers et al., 2007). In the current study, the aerosol route administration seemed less sensitive to detect differences in reactivity to methacholine than the IV one.

Albuterol is a potent bronchodilator with beta 2-specific adrenergic agonist activity (Petruska et al., 1997). Its use has been reported in dogs and monkeys (Authier et al., 2009; Chapman et al., 2005), but not in Göttingen minipigs. In our study, albuterol administration resulted in a significant reduction in the slopes of R_{rs} and E_{rs} methacholine response curves for dogs and monkeys. Exposure to methacholine before or after albuterol induced only modest effects on R_{rs} or E_{rs} in minipigs. The post-albuterol slope variation was higher in monkeys and dogs than in minipigs, suggesting a higher reactivity of minipigs to post-albuterol methacholine. Indeed, it could be hypothesised that either minipigs kept a higher reactivity to post-albuterol methacholine compared to both other species (as observed with the response to IV injection of methacholine), or that minipigs were less sensitive to albuterol for counteracting the bronchoconstrictive property of methacholine, or both. However, there was no difference between species when comparing the methacholine slopes before albuterol. The porcine species has predominant expression of β_1-adrenergic receptors in the lungs over β_2 adrenergic receptors (Liang & Mills, 2002) which may also, at least partially, explain the lack of response to albuterol in the current study. In contrast, dogs have largely predominant expression of β_2-adrenergic receptors in the lungs over β_2 (Manalan, Besch, & Watanabe, 1981). In addition, blood gas analysis performed in dogs did not reveal any significant effect of bronchocontriction and associated ventilation changes. Consistently, there was no statistical difference in PC_{200} values for resistance in minipigs between pre- and post-albuterol treatment. A similar result was also obtained in monkeys, however, as can be appreciated in Fig. 1, that observation was mainly driven by the presence of an extremely responsive subject (subject A) to albuterol administration (PC_{200} pre- and post-albuterol: 1.72 vs 22.63 mg/mL), which increased variability and challenged the statistical comparison of means. In fact, as can be observed in Fig. 1 for monkeys as well as for dogs, all but one subject required more challenges to double their resistance after albuterol administration, suggesting that the bronchodilator produced a physiological switch in methacholine sensitivity. In minipigs, the number of challenges required to double baseline R_{rs} remained unchanged after albuterol administration. These results therefore confirmed the poor sensitivity of this methodology in safety pharmacology as previously reported (Authier et al., 2008) even at much higher methacholine doses as used in the current study.

From a respiratory mechanics point of view, the results from the current study suggest similarities between dogs and monkeys in terms of their reactivity to methacholine-induced bronchoconstriction and sensitivity to albuterol-mediated bronchodilation. Yet, the ventilatory response to bronchoconstriction in the monkey is closer to humans and also present relatively important variations in the response to methacholine between individuals (Wilson, Phagoo, & Silverman, 1991). Such similarities, including the nature of the respiratory changes between Cynomolgus monkeys and humans, were previously identified in conscious animals with spirometry (Authier et al., 2009). These pharmacodynamic similarities correlate with anatomic, physiologic and histologic characteristics of monkeys and dogs that resemble humans while minipigs 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 pigs have only three generations of bronchiole (Rogers et al., 2008). The number of alveolar pores, which facilitates collateral ventilation, is similar in dog, monkey and human alveoli and lower in minipigs (Robinson, 1982; Rogers et al., 2008). The absence of collateral ventilation in pigs may predispose to small airway obstruction, with decreased vital capacity and increased functional residual capacity. Submucosal glands are observed throughout the bronchial tree of dogs (Takenaka et al., 1996), monkeys (El-Bermani & Grant, 1975), humans (Scott, 1973) and minipigs (Rogers et al., 2008).

Will anatomical and physiological similarities between monkeys and humans translate into increased predictive value in drug development 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 during design of non-clinical safety testing and evaluation of the translational value of these species with a much broader range of respiratory modulators would be needed to address these questions. The use of a model such as the one described in the current study also needs to be put in perspective as drug safety testing is facing uncertainty relative to effective/active dose levels of the test agents which may increase the experimental iterations and the number of doses that are required to rule-out or confirm effects on respiratory mechanics. It remains that dose levels used in the current study induced reliable bronchoconstriction, bronchodilation and ventilatory alterations given the well-characterized positive control drugs that were selected, and this study supports the use of the three species for follow-up respiratory safety pharmacology investigations.

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References

Authier, S., Gervais, J., Fournier, S., Gauvin, D., Maghezzi, S., & Troncy, E. (2011). Cardiovascular and respiratory safety pharmacology in Göttingen minipigs: Pharmacological characterization. *Journal of Pharmacological and Toxicological Methods*, *64*, 53–59.

Authier, S., Legaspi, M., Gauvin, D., Chaurand, F., Fournier, S., & Troncy, E. (2008). Validation of respiratory safety pharmacology models: conscious and anesthetized beagle dogs. *Journal of Pharmacological and Toxicological Methods*, *57*, 52–60.

Authier, S., Legaspi, M., Gauvin, D., & Troncy, E. (2009). Respiratory safety pharmacology: positive control drug responses in Sprague-Dawley rats, Beagle dogs and cynomolgus monkeys. *Regulatory Toxicology and Pharmacology*, *55*, 229–235.

Chapman, R. W., Skeans, S., Lamca, J., House, A., Hey, J. A., & Celly, C. (2005). Effect of histamine, albuterol and deep inspiration on airway and lung tissue mechanics in cynomolgus monkeys. *Pulmonary Pharmacology & Therapeutics*, *18*, 243–249.

Dungworth, D. L., Castleman, W. L., Chow, C. K., Mellick, P. W., Mustafa, M. G., Tarkington, B., et al. (1975). Effect of Ambient Levels of Ozone on Monkeys. *Federation Proceedings*, *34*, 1670–1674.

Dybas, J. M., Andresen, C. J., Schelegle, E. S., McCue, R. W., Callender, N. N., & Jackson, A. C. (2006). Deep-breath frequency in bronchoconstricted monkeys (Macaca fascicularis). *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *100*, 786–791.

El-Bermani, A. W., & Grant, M. (1975). Acetylcholinesterase-positive nerves of the rhesus monkey bronchial tree. *Thorax*, *30*, 162–170.

Kleinsasser, A., Olfert, I. M., Loeckinger, A., Prisk, G. K., Hopkins, S. R., & Wagner, P. D. (2007). Tidal volume dependency of gas exchange in bronchoconstricted pig lungs. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *103*, 148–155.

Liang, W., & Mills, S. E. (2002). Quantitative analysis of beta-adrenergic receptor subtypes in pig tissues. *Journal of Animal Science*, *80*(4), 963–970.

Lindgren, S., Bass, A. S., Briscoe, R., Bruse, K., Friedrichs, G. S., Kallman, M. J., et al. (2008). Benchmarking Safety Pharmacology regulatory packages and best practice. *Journal of Pharmacological and Toxicological Methods*, *58*, 99–109.

Madwed, J. B., & Jackson, A. C. (1997). Determination of airway and tissue resistances after antigen and methacholine in nonhuman primates. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *83*, 1690–1696.

Manalan, A. S., Besch, H. R., Jr., & Watanabe, A. M. (1981). Characterization of [3H](+/-) carazolol binding to beta-adrenergic receptors. Application to study of beta-

adrenergic receptor subtypes in canine ventricular myocardium and lung. *Circulation Research*, *49*(2), 326–336.
McGovern, T. K., Robichaud, A., Fereydoonzad, L., Schuessler, T. F., & Martin, J. G. (2013). Evaluation of Respiratory System Mechanics in Mice using the Forced Oscillation Technique. *Journal of Visualized Experiments*, *75*, e50172.
Petruska, J. M., Beattie, J. G., Stuart, B. O., Pai, S., Walters, K. M., Banks, C. M., et al. (1997). Cardiovascular effects after inhalation of large doses of albuterol dry powder in rats, monkeys, and dogs: A species comparison. *Fundamental and Applied Toxicology*, *40*, 52–62.
Robinson, N. E. (1982). Some Functional Consequences of Species-Differences in Lung Anatomy. *Advances in Veterinary Science and Comparative Medicine*, *26*, 1–33.
Rogers, C. S., Abraham, W. M., Brogden, K. A., Engelhardt, J. F., Fisher, J. T., McCray, P. B. Jr, et al. (2008). The porcine lung as a potential model for cystic fibrosis. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, *295*, L240–L263.
Saetta, M., Kim, W. D., Izquierdo, J. L., Ghezzo, H., & Cosio, M. G. (1994). Extent of Centrilobular and Panacinar Emphysema in Smokers Lungs - Pathological and Mechanical Implications. *European Respiratory Journal*, *7*, 664–671.
Scott, K. W. (1973). An autopsy study of bronchial mucous gland hypertrophy in Glasgow. *The American Review of Respiratory Disease*, *107*, 239–245.
Shalaby, K. H., Gold, L. G., Schuessler, T. F., Martin, J. G., & Robichaud, A. (2010). Combined forced oscillation and forced expiration measurements in mice for the assessment of airway hyperresponsiveness. *Respiratory Research*, *11*, 82.
Sly, P. D., & Lanteri, C. J. (1991). Partitioning of pulmonary responses to inhaled methacholine in puppies. *Journal of Applied Physiology*, *71*, 886–891.
Sly, P. D., Willet, K. E., & Habre, W. (1998). Environmental effects on pulmonary mechanics and the response to inhaled methacholine. *Pediatric Pulmonology*, *25*, 332–337.
Takenaka, S., Heini, A., Ritter, B., & Heyder, J. (1996). Morphometric evaluation of bronchial glands of beagle dogs. *Toxicology Letters*, *88*, 279–285.
Takenaka, S., Heini, A., Ritter, B., & Heyder, J. (1998). The respiratory bronchiole of beagle dogs: structural characteristics. *Toxicology Letters*, *96–7*, 301–308.
U. S. Food and Drug Administration (2001). *Guidance for Industry: Safety Pharmacology Studies for Human Pharmaceuticals (S7A)*.
U. S. Food and Drug Administration (2005). *Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers*.
Van Scott, M. R., Hooker, J. L., Ehrmann, D., Shibata, Y., Kukoly, C., Salleng, K., et al. (2004). Dust mite-induced asthma in cynomolgus monkeys. *Journal of Applied Physiology*, *96*, 1433–1444.
Wagers, S. S., Haverkamp, H. C., Bates, J. H., Norton, R. J., Thompson-Figueroa, J. A., Sullivan, M. J., et al. (2007). Intrinsic and antigen-induced airway hyperresponsiveness are the result of diverse physiological mechanisms. *Journal of Applied Physiology*, *102*, 221–230.
Walker, J. K., Kraft, M., & Fisher, J. T. (2012). Assessment of murine lung mechanics outcome measures: alignment with those made in asthmatics. *Frontiers in Physiology*, *3*, 491.
Wilson, N. M., Phagoo, S. B., & Silverman, M. (1991). Use of transcutaneous oxygen tension, arterial oxygen saturation, and respiratory resistance to assess the response to inhaled methacholine in asthmatic children and normal adults. *Thorax*, *46*, 433–437.