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Original article

Non-invasive measure of respiratory mechanics and conventional respiratory parameters in conscious large animals by high frequency Airwave Oscillometry

Leanne Bassett [a], Eric Troncy [b], Annette Robichaud [c], Thomas F. Schuessler [c,d], Mylène Pouliot [a], Alexis Ascah [a], Simon Authier [a,b,*]

[a] CIToxLAB North America, 445 Armand Frappier, Laval, QC, Canada, H7V 4B3
[b] Faculty of Veterinary Medicine, University of Montreal, P.O. Box 5000, St-Hyacinthe, QC, Canada, J2S 7C6
[c] SCIREQ Scientific Respiratory Equipment Inc., 6600 St-Urbain, Montreal, QC, Canada, H2S 3G8
[d] THORASYS Thoracic Medical Systems Inc., 6600 rue St-Urbain, Montreal, QC, Canada, H2S 3G8

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ABSTRACT

Introduction: A number of drugs in clinical trials are discontinued due to potentially life-threatening airway obstruction. As some drugs may not cause changes in core battery parameters such as tidal volume (V_t), respiratory rate (RR) or minute ventilation (MV), including measurements of respiratory mechanics in safety pharmacology studies represents an opportunity for design refinement. The present study aimed to test a novel non-invasive methodology to concomitantly measure respiratory system resistance (R_{rs}) and conventional respiratory parameters (V_t, RR, MV) in conscious Beagle dogs and cynomolgus monkeys. **Methods:** An Airwave Oscillometry system (*tremoFlo*; THORASYS Inc., Montreal, Canada) was used to concomitantly assess R_{rs} and conventional respiratory parameters before and after intravenous treatment with a bronchoactive agent. Respiratory mechanics measurements were performed by applying a short (*i.e.* 16 s) single high frequency (19 Hz) waveform at the subject's airway opening *via* a face mask. During measurements, pressure and flow signals were recorded. After collection of baseline measurements, methacholine was administered intravenously to Beagle dogs ($n = 6$) and cynomolgus monkeys ($n = 4$) at 8 and 68 μg/kg, respectively. **Results:** In dogs, methacholine induced significant increases in V_t, RR and MV while in monkeys, it only augmented RR. A significant increase in R_{rs} was observed after methacholine administration in both species with mean percentage peak increases from baseline of 88 (53)% for dogs and 28 (16)% for cynomolgus monkeys. **Conclusion:** Airwave Oscillometry appears to be a promising non-invasive methodology to enable respiratory mechanics measurements in conscious large animals, a valuable refinement in respiratory safety pharmacology.

1. Introduction

In the realm of safety pharmacology, evaluation of respiratory function follows the ICH S7A Guideline (Anon, 2001), which states that the latter be included in core battery studies during the pre-clinical drug development phase, as respiratory distress can have life-threatening consequences. While most safety pharmacology studies (*i.e.* between 98 and 100%) include measurements of respiratory function parameters—tidal volume (V_t), respiratory rate (RR) and minute ventilation (MV)—acquired with techniques such as plethysmography, only 39% report inclusion of respiratory mechanics parameters such as respiratory system resistance (R_{rs}) and compliance (Legaspi et al., 2010; Lindgren et al., 2008). Monitoring of respiratory mechanics in safety pharmacology studies was shown to be relevant in drug development as changes in these parameters are not always reflected in breathing patterns, typically assessed through conventional respiratory function parameters (Murphy, 2013; Savoy, Allgöwer, Courteheuse, & Junod, 1984). A number of drugs, including antibiotic, anti-inflammatory and chemotherapeutic agents, have been known to cause bronchoconstriction, thereby potentially increasing airway resistance (Murphy, 2005). As presented by Murphy (2013), approximately 29% of respiratory difficulties encountered in human clinical trials are due to airway obstruction, further increasing the relevance of respiratory mechanics monitoring in early drug development.

Reliable, though invasive, advanced methodologies to obtain measurements of respiratory mechanics have been demonstrated (Truchetti

* Corresponding author at: CIToxLAB North America, 445 Armand Frappier, Laval, Quebec, Canada, H7V 4B3.
E-mail address: authiers@ca.citoxlab.com (S. Authier).

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et al., 2014). A non-invasive technique of measurements would hold the potential to detect drug-induced respiratory effects in conscious animals without the confounding variable introduced by anesthesia. Airwave Oscillometry is a novel, non-invasive technique for measuring R_{rs} in conscious subjects that also captures the traditional respiratory function parameters (*i.e.* RR, V_t and MV). In this technique, the resistance of the respiratory system (which includes the lung, upper airways and chest wall resistances) is obtained by gently forcing low amplitude pressure- or volume-driven oscillations into the subject's respiratory airflow while capturing the response of the respiratory system. This technique, which is referred to as the Forced Oscillation Technique (FOT), produces precise measurements of R_{rs} (Kaminsky, 2012; Oostveen et al., 2003) and has been used successfully in human subjects with varying severities of asthma or chronic obstructive pulmonary disease (Kaminsky, 2012).

The current study aimed to characterize R_{rs} in conscious Beagle dogs and cynomolgus monkeys concomitantly with conventional respiratory function parameters using Airwave Oscillometry as a non-invasive tool to identify drug-induced respiratory changes.

2. Materials and methods

2.1. Statement on use and care of animals.

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 US National Institutes of Health (NIH Publication, revised 2011). CiToxLAB North America's facility is AAALAC accredited. All procedures were conducted as per Standard Operating Procedures (SOPs) in place.

2.2. Animal housing

2.2.1. Beagle dogs

Six (6) male Beagle (*Canis familiaris*) dogs were selected for non-invasive monitoring of respiratory parameters (V_t, RR, MV and R_{rs}). The animals were between 1 and 2 years old and weighed between 7.1 and 10.7 kg. The animal room environment was controlled (temperature 21 ± 3 °C, humidity 30–70%, 12 h light, 12 h dark, 10–15 air changes per hour) and temperature and relative humidity were monitored continuously. A standard certified commercial dog chow (Harlan Teklad Certified 25% Lab Dog Diet #8727C^{TM}, Harlan Teklad, Madison, WI, USA) was available daily.

2.2.2. Cynomolgus monkeys

Four (4) male cynomolgus (*Macaca fascicularis*) monkeys were selected for non-invasive monitoring of respiratory parameters (V_t, RR, MV and R_{rs}). Monkeys were 3–4 years old and weighed between 3.0 and 4.3 kg. The animal room environment was controlled (temperature 21 ± 3 °C, humidity 30–70%, 12 h light, 12 h dark, 10–15 air changes per hour) and temperature and relative humidity were monitored continuously. A standard certified commercial primate chow (Certified Hi-Fiber Primate Diet 7195C^{TM}, Harlan Teklad, Madison, WI, USA) was available to each monkey twice daily.

2.3. Experimental methods

Animals were acclimated to the respiratory monitoring equipment on three occasions prior to data collection with sessions of increasing duration and using positive reinforcement. Conscious animals were restrained using a hammock type restrainer (*e.g.* sling from Lomir Biomedical, Notre-Dame-de-L'île-Perrot, QC, Canada). The respiratory monitoring device was calibrated daily by measurement of a mechanical test load that is included with the system. Respiratory system resistance and conventional respiratory parameters (RR, V_t, MV) were evaluated concomitantly in conscious Beagle dogs and cynomolgus monkeys before and after an intravenous injection of methacholine (Sigma-Aldrich, Oakville, ON, Canada), a bronchoconstrictive agent. Dose levels were, respectively, 8 and 68 μg/kg for Beagle dogs and cynomolgus monkeys. Monitoring was performed non-invasively using high frequency Airwave Oscillometry (tremoFlo™ Airwave Oscillometry System, THORASYS Thoracic Medical Systems Inc., Montreal, Canada) (Fig. 1). More specifically, a 16 second long, volume-driven (peak-to-peak amplitude of 6 mL), single high frequency (19 Hz) waveform was gently delivered at each time point into the subject's airway opening *via* a face mask (LNR, Ville St-Laurent, QC, Canada). The oscillation was delivered using a mechanical system inducing a 19 Hz movement to one of the two pneumotachometers included in the measuring system. The duration of monitoring (*i.e.* 16 s) was selected based on prior analysis of respiratory data from various sources and the anticipated shortest length of data recording that would provide accurate and reproducible estimations of respiratory function. During measurements, pressure and flow signals were recorded. R_{rs} at 19 Hz was calculated directly in the device operating software (THORASYS Thoracic Medical Systems Inc., Montreal, Canada). Briefly, pressure and flow signals acquired at a 256 Hz sampling rate were bandpass filtered, using a 4th order Bessel filter, and Fourier transformed. The respiratory system input impedance (Z_{rs}), or the transfer function between the input and output signals, was then determined over 1 second interval with a 50% overlap and expressed as the average of individual windows for the measurement. R_{rs} was automatically extracted from the real part of Z_{rs} at 19 Hz. Datasets having a coefficient of determination below 0.9 were automatically rejected by the software. RR and V_t were extracted for the recorded signals directly in the operating software while MV was determined from exported parameters. Measurements were taken approximately every minute for 15 min to establish stable and reproducible baseline values, and up to 10 min following the induction of the bronchoconstrictor challenge. In between measurements, animals were disconnected from the device and face mask, which greatly facilitated data capture in these conscious animals. Individual subject results are presented at baseline and at peak bronchoconstriction. Differences between experimental conditions in the same group were determined by paired *t*-test, considering two-sided $p < 0.05$ as statistically significant (GraphPad Prism version5; GraphPad Software, San Diego, CA, USA). Differences between groups were tested by *t*-test. Data are presented as mean (standard deviation).

3. Results

Baseline values and methacholine-induced changes for R_{rs} at 19 Hz and conventional respiratory function parameters in Beagle dogs and cynomolgus monkeys are presented in Fig. 2. Intravenous methacholine

Fig. 1. Schematic representation of the tremoFlo^{TM} Airwave Oscillometry System device used for the combined, non-invasive measurements of respiratory system resistance and conventional respiratory function parameters in conscious subjects.

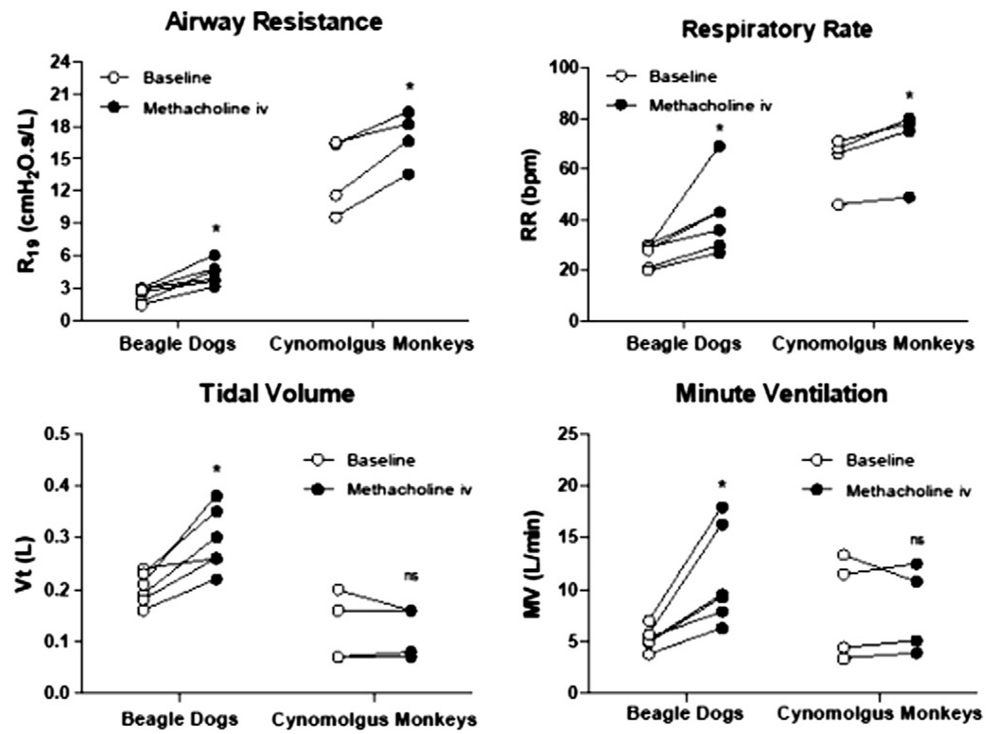

Fig. 2. Methacholine induced changes on respiratory system resistance at 19 Hz (R_{19}), respiratory rate (breaths per minute, bpm), tidal volume (liter, L) and minute ventilation (liter per minute, L/min) in Beagle dogs ($n = 6$; 8 μg/kg) and cynomolgus monkeys ($n = 4$; 68 μg/kg). *: indicates a p-value <0.05 for two-sided paired t-test.

significantly increased V_t, RR and MV in Beagle dogs ($p < 0.05$) with mean peak increases of +46 (25)%, +56 (41)% and +106 (54)%, respectively. Of the previously mentioned parameters, only RR was significantly increased in cynomolgus monkeys ($p < 0.05$) following administration of methacholine, with a mean peak increase of +12 (5)%.

The mean baseline values for R_{rs} at 19 Hz were 2.45 (0.67) cmH_20 s/L and 13.53 (3.46) cmH_20 s/L for Beagle dogs and cynomolgus monkeys, respectively. A significant increase in that parameter was noted following administration of methacholine in Beagle dogs and cynomolgus monkeys with mean peak increases of +88 (53)% and +28 (16)%, respectively, when compared to baseline values ($p < 0.05$).

4. Discussion

Evaluation of respiratory mechanics in large animals is of prime interest for detection of drug-induced respiratory changes in drug development. Measurements can be conducted under anesthesia, which can significantly alter respiratory patterns and lung function. In contrast, other methodologies use surgical probe implantation which is invasive and may interfere with histopathology evaluations. In the current study, Airwave Oscillometry was used to non-invasively measure respiratory system resistance concomitantly with conventional respiratory function parameters in conscious Beagle dogs and cynomolgus monkeys before and after methacholine administration. The results suggest that Airwave Oscillometry is a valuable translational tool for non-invasive measurement of respiratory mechanics in large animal species.

As previously reported, baseline airway resistance showed higher values in non human primates when compared to dogs (Truchetti et al., 2014). This could represent an adaptation from natural selection where dogs require a highly efficient respiratory system in various situations requiring the animal to fight and/or run. In contrast, non human primates require a smaller respiratory system relative to body mass to enable efficient vertical escape (*e.g.* tree climbing) and consequently respiratory components may not be optimized to minimize resistance as noted in the dog. Significant increases in R_{rs} at 19 Hz and RR were observed in Beagle dogs and cynomolgus monkeys after methacholine intravenous injection, although increases in V_t and MV were only noted in Beagle dogs. The lack of a significant increase in V_t and MV in cynomolgus monkeys following administration of methacholine in the present study supports the inclusion of R_{rs} as a valuable parameter in respiratory safety pharmacology. The resistance of the respiratory system, as measured in the present study, includes the lung, upper airways and chest wall resistances. However, since at high frequencies R_{rs} is dominated by the contribution of the upper and central airways, R_{rs} measurements taken at 19 Hz represent predominantly the airway resistance.

The effect of methacholine on respiratory function parameters in dogs and non-human primates has been previously characterized (Authier, Legaspi, Gauvin, & Troncy, 2009), confirming the observed pharmacological effects. As a bronchoconstrictor, methacholine is expected to increase resistance due to the stimulation of muscarinic, postganglionic parasympathetic receptors (Birnbaum & Barreiro, 2007). Methacholine has been shown to induce a decrease in both V_t and MV in humans (Fujimori, Satoh, & Arakawa, 1996), similar to results obtained in cynomolgus monkeys previously (Authier et al., 2009). In the present study, methacholine was associated with a mild increase in RR. The results of the present study concur with those described by Murphy (2013), in that changes in R_{rs} did not necessarily translate in alteration to breathing patterns following administration of methacholine. This finding points to the relevance of including respiratory mechanics parameters, such as R_{rs}, in respiratory safety pharmacology studies. The FOT can be combined to conventional spirometry for the concomitant acquisition of supplemental, relevant parameters in core battery studies involving large animal models. R_{rs} depends on lung volume and the current study did not measure or control for this parameter which represents a potential limitation. Airway resistance (R_{aw}) is inversely related to lung volume. An increase in V_t was noted following methacholine administration in dogs which would be expected to decrease R_{aw}. As the effects on R_{aw} showed the opposite (an increase of this parameter), the theoretical limitation associated with lung volume changes was not physiologically significant.

In a recent industry survey (Authier, Vargas, Curtis, Holbrook, & Pugsley, 2013), respondents indicated that rats (37%) and large animals

(48%) were used in studies adding respiratory safety pharmacology endpoints to investigative toxicology studies. While the rat remains the most frequently used species for core battery respiratory studies (Lindgren et al., 2008), the use of large animals for respiratory assessments has increased in recent years. Regulators require that respiratory safety assessments be conducted in a relevant species which often precludes the use of rats for biologics. Owing to the closer homology to humans and similarities in receptor expression and affinity, respiratory safety pharmacology studies utilizing large animals may continue to growth (Willyard, 2009). Interestingly, the number of Biological License Application (BLA) approvals has increased from 9 in 2010 to 16 in 2013 (U.S. Food Drug Administration, 2013). As outlined in ICH S6 (R1) Guideline (Anon, 2011), safety pharmacology investigations are required in safety assessments of biotechnology derived pharmaceuticals. While 68% of safety pharmacologists report using rats in studies where respiratory measurements were added to toxicology studies with new chemical entities (NCEs), only 40% include rats in studies with biological agents (Authier et al., 2013) This is likely an expected consequence of the more important phylogenetic differences between humans and rodents when compared to large animals justifying the use of the latter for biologics. According to the same survey, more non-human primates were used in studies with biological agents when compared to NCEs (76% and 53%, respectively). This, taken with the fact that approximately 95% of respiratory system endpoints added to toxicology studies were considered acceptable by regulatory authorities (Authier et al., 2013), suggests that a non-invasive method offering a "broader panel" of respiratory measurements in large animals would represent a valuable tool. Despite the advantages of a non-invasive methodology for R_{rs} monitoring, potential limitations including the impact of stress due to restraint on the respiratory measurements, the impact of intermittent measurements on data variability, and the inability to monitor during the sleep state need to be considered during study design. Globally, the methodology offers non-invasive airway resistance which has the potential to enhance the relevance of respiratory measurements in a number of non-clinical safety testing studies including after repeat dose administration.

Conflicts of interest

None of the authors have any conflicts of interest, other than their employment in commercial companies or contract research organizations.

References

Anon (2001). *ICH Expert Working Group (Safety) Guidance for Industry—S7A Safety Pharmacology Studies for Human Pharmaceuticals.*

Anon (2011). *ICH-S6 (R1) preclinical safety evaluation of biotechnology-derived pharmaceuticals.*

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.

Authier, S., Vargas, H. M., Curtis, M. J., Holbrook, M., & Pugsley, M. K. (2013). Safety pharmacology investigations in toxicology studies: An industry survey. *Journal of Pharmacological and Toxicological Methods*, *68*, 44–51.

Birnbaum, S., & Barreiro, T. J. (2007). Methacholine challenge testing: Identifying its diagnostic role, testing, coding, and reimbursement. *Chest*, *131*, 1932–1935.

Fujimori, K., Satoh, M., & Arakawa, M. (1996). Ventilatory response to continuous incremental changes in respiratory resistance in patients with mild asthma. *Chest*, *109*, 1525–1531.

Kaminsky, D. A. (2012). What does airway resistance tell us about lung function? *Respiratory Care*, *57*, 85–99.

Legaspi, M., Authier, S., Gauvin, D., Moreau, M., Beauchamp, G., Chaurand, F., et al. (2010). Respiratory safety pharmacology: Concurrent validation of volume, rate, time, flow and ratio values in conscious male Sprague–Dawley rats. *Regulatory Toxicology and Pharmacology*, *58*, 444–450.

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.

Murphy, D. J. (2005). Comprehensive non-clinical respiratory evaluation of promising new drugs. *Toxicology and Applied Pharmacology*, *207*, 414–424.

Murphy, D. J. (2013). Respiratory safety pharmacology—Current practice and future directions. *Regulatory Toxicology and Pharmacology*. http://dx.doi.org/10.1016/j.yrtph.2013.11.010.

Oostveen, E., MacLeod, D., Lorino, H., Farre, R., Hantos, Z., Desager, K., et al. (2003). The forced oscillation technique in clinical practice: Methodology, recommendations and future developments. *European Respiratory Journal*, *22*, 1026–1041.

Savoy, J., Allgöwer, E., Courteheuse, C., & Junod, A. F. (1984). Ventilatory response to bronchospasm induced by methacholine and histamine in man. *Respiration Physiology*, *56*, 195–203.

Truchetti, G., Troncy, E., Gold, L., Robichaud, A., Maghezzi, S., & Authier, S. (2014). Respiratory mechanics: Comparison of Beagle dogs, Göttingen minipigs and cynomolgus monkeys. *Journal of Pharmacological and Toxicological Methods*. http://dx.doi.org/10.1016/j.vascn.2014.03.171 (Current Issue).

U.S. Food Drug Administration (2013). Biological approvals by year. Available from. http://www.fda.gov/BiologicsBloodVaccines/DevelopmentApprovalProcess/BiologicalApprovalsbyYear/default.htm.

Willyard, C. (2009). Profit-hungry pharma sees biotechs as ripe for the picking. *Nature Medicine*, *15*, 466.