Journal of the American Association for Laboratory Animal Science
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Vol 47, No 5
September 2008
Pages 52–55

Whole-body Plethysmography in African Green Monkeys (*Chlorocebus aethiops*) with and without Jackets

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Indwelling central venous catheters are often used to facilitate frequent phlebotomy while minimizing stress and anesthetic effects on animals. However, nonhuman primates with central venous catheters must wear protective jackets. Jackets routinely are removed for aerosol exposure to agents and respiratory measurements by whole-body plethysmography (WBP) because of the potentially confounding effects of jackets on these procedures. However, removing the jacket may dislodge the catheter, making it unusable. Using each animal as its own control, we tested 12 African green monkeys to determine whether minute volume, tidal volume, respiratory rate, or accumulated volume measurements by WBP differed depending on whether the animal wore a protective jacket or not. We found no statistical differences in any measured respiratory parameter and concluded that the jackets could be left in place on the animal while undergoing plethysmography without compromising the calculations for determining the inhaled dose of aerosolized agent. In addition, this study revealed no obvious contraindications to leaving the jacket in place in other nonhuman primate species, provided that the jacket fits appropriately and that plethysmography is performed correctly.

Abbreviations: AGM, African green monkey; WBP, whole-body plethysmography

The inhalational route is widely accepted as presenting the most likely risk for exposure to biothreat agents, either in the battlefield or from a terrorist attack.[5] Well-characterized animal models of disease associated with inhalational exposure to identified threat agents are crucial in the study of these biothreat agents because exposing humans to pathogens that may cause fatal or debilitating disease is unethical. The development of animal models will allow their use for licensing of vaccines and therapeutic drugs in compliance with the FDA Animal Rule (21 CFR 314), because future efficacy studies will produce data relevant to licensure of new medical countermeasures. Nonhuman primates are closely related to humans phylogenetically[17] and remain the best predictors of success for vaccines and therapies in humans.[12] Models involving African green monkeys are especially desirable because, unlike rhesus and cynomolgus macaques, green monkeys are not at risk of transmitting *Cercopithecine herpesvirus 1* to caretakers and laboratory technicians.[9] Green monkey models for many biothreat agents have been developed and are widely used.[1,2,7,9,19]

In our facility, nonhuman primates are exposed to aerosolized biothreat agents by use of a head-only exposure chamber contained in a Class III biological safety cabinet. The aerosol is controlled by an automated biological exposure system, which has been described.[8] The inhaled dose is obtained most accurately from calculations using the individual minute volume for each animal. Previous studies have shown that mathematical predictors of minute volume are not appropriate for determining minute volume under ketamine, acepromazine–ketamine, or tiletamine–zolazepam anesthesia.[3] In our facility, 3 min of whole-body plethysmography (WBP) is performed to determine the minute volume for each anesthetized nonhuman primate immediately prior to aerosol exposure.[3,8,12-14]

Characterization of animal models is becoming increasingly sophisticated. In many studies, frequent phlebotomy is required to obtain blood samples for the evaluation of biomarkers of infection or assessment of host immune responses. To eliminate the confounding variables associated with repeated capture and anesthetic events, on a disease course, investigators now frequently use indwelling central venous catheters to obtain these samples. After surgical implantation of central venous catheters, nonhuman primates must wear nonrestraining jackets to protect the catheter from the cage environment and from the animals themselves. The catheter is routed out the back of the jacket and through a flexible stainless steel tether, which attaches to a swivel on the cage wall. The use of a catheter–jacket–tether system in nonhuman primates allows for repeated blood collection and fluid administration without the use of anesthesia and without inducing capture-related stress. This system eliminates the effects that these 2 stressors have on body temperature, activity, and appetite, which are key clinical assessments in many infectious disease models.[6]

Historically, plethysmography and aerosol exposures in our facility have been performed after removal of the jackets. Although the jackets are fit-tested individually to ensure that they do not restrict the movement and behavior of the animals wearing them, whether wearing the jacket in the plethysmography box would alter the measured parameters was unknown. If respiratory data were skewed, the minute volume would be inaccurate, leading to incorrect calculation of presented dose. However, on several occasions, inadvertent manipulation of the unprotected catheter during these procedures directly resulted in catheter failure.

Received: 19 March 2008. Revision requested: 15 April 2008. Accepted: 9 May 2008.
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The objective of this study was to determine whether results of WBP performed on a nonhuman primate wearing a nonrestraining jacket differed significantly from those from the same, but nonjacketed, animal. The following respiratory variables were compared: minute volume, tidal volume, respiratory rate, and accumulated volume (cumulative sum of all tidal volumes measured during the collection period). To control for pulmonary differences between individual nonhuman primates, comparisons were made by using data from the same animal. During week 1, the animals wore the jackets; the next week, unjacketed animals were analyzed. A group of 12 African green monkeys was evaluated both with and without the jacket for plethysmography measures.

Materials and Methods

Animals. Twelve African green monkeys (*Chlorocebus aethiops*), 6 male and 6 female and ranging in weight from 3.9 to 5.5 kg, were assigned from the colony at our institution. Each animal was clinically normal on physical examination and was seronegative for measles virus, *Cercopithecine Herpesvirus* 2, SIV, and simian T-cell leukemia virus. No intestinal parasites were detected on fecal examination. All of the animals were housed in 4.5-ft^2 cages with 4 cages per rack (Allentown Caging Equipment, Allentown, NJ), and environmental conditions were maintained as recommended in the *Guide for the Care and Use of Laboratory Animals*[10] (temperature, 16 to 29 °C; humidity, 30% to 70%; and 12:12-h light:dark cycle). Animals were fed a standard primate diet (diet 8714, Harlan Teklad, Madison, WI) supplemented with fruit and other food treats. Fresh water, provided ad libitum, was chlorinated at the municipal level and filtered (Edstrom Industries, Waterford, WI). Environmental enrichment (Challenge Ball, Kong, and Hercules Dental Device, Bio-Serv, Frenchtown, NJ) was provided, and cages were arranged so that the animals were facing each other across the room.

All described procedures were performed as part of an animal research protocol reviewed and approved by our facility's institutional animal care and use committee. Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in *the Guide for the Care and Use of Laboratory Animals*.[10] The facility is fully accredited by AAALAC.

Experimental design. Food was withheld from all study animals for approximately 12 h before sedation. Before each plethysmographic measurement, the monkeys were sedated with a mixture of ketamine and acepromazine at a dose that delivered 9 mg ketamine HCl and 0.1 mg acepromazine maleate per kilogram of body weight. Each anesthetic injection was given intramuscularly in the caudal thigh.

Each assessment consisted of collection of respiratory parameters by WBP (Buxco Biosystem XA software, Buxco Electronics, Wilmington, NC). The WBP system was calibrated each day before collecting respiratory data. Animals were anesthetized, weighed, and placed in the plethysmograph box for measurement of respiratory parameters. Seven days later, the animals were anesthetized, weighed, and fitted with an appropriately sized nonrestraining jacket (Primate Jacket, Lomir Biomedical, Malone, NY) before plethysmography. Monkeys weighing less than 5.0 kg were fitted with a medium jacket, and those weighing 5.0 kg or more were fitted with a large jacket. Plethysmography was performed as previously described,[3] with the exception that a foam collar replaced the rubber collar previously used and a newer version of the software was used. Briefly, the monkeys were placed into a medium acrylic plethysmograph box in dorsal recumbency, with the animal's head protruding from the box and resting on a pillow (Figure 1). The top of the box was secured, and data were collected and recorded at 10-s intervals over 3 min. The final value for each measured parameter was an average value over the 3 min of collection. Each animal was evaluated in two iterations of the plethysmography experiment; each iteration consisted of both jacketed and nonjacketed assessments. The second iteration was started 3 wk after the first iteration.

Statistical analysis. Each parameter was analyzed with and without jackets by paired *t* tests using each animal as its own control.[18] Statistics software (SAS Version 9.1.3 and SAS OnlineDoc Version 9, SAS Institute, Cary, NC, 2003) was used for all analyses at the 2-tailed 95% confidence level for all tests. The threshold for statistical significance was a *P* value of 0.05.

Results

The results of iterations 1 and 2 are presented in Tables 1 and 2, respectively. Comparing values for each condition in each green monkey controlled for interanimal variability due to basic expected physiologic differences. Within each iteration, all respiratory parameters evaluated were internally consistent. The presence or absence of the jacket did not significantly alter accumulated volume, minute volume, respiratory rate, or tidal volume measurements by WBP.

Discussion

Our study shows that WBP can successfully be performed on African green monkeys wearing nonrestraining protective jackets. The lack of significant changes in measured respiratory parameters for anesthetized green monkeys wearing jackets indicates that appropriately sized protective jackets do not restrict the animals' breathing and do not otherwise interfere with collection of respiratory data. Although this study exclusively involved African green monkeys, similar results may be possible in other commonly used species, provided the jackets are appropriately fitted.

Accurate collection of respiratory data by plethysmography depends on maintaining a seal on the box throughout the collection period. To properly seal the plethysmography box, the animal must be positioned so that the collar of the protective jacket is entirely within the box and not protruding through the head opening and dental dam. Although this positioning was easily accomplished during our study, which involved green monkeys weighing 3.7 to 5.5 kg, correct positioning may be difficult on a small monkey with a shorter neck. Although the minimum size requirement for an African green monkey to undergo plethysmography while wearing a jacket is unknown, if a much smaller animal were used, custom jackets with shorter collars likely would be necessary.

To control any variables that might have been introduced as a result of anesthesia, we attempted to standardize the anesthetic depth between jacketed and nonjacketed green monkeys during plethysmography. Within each iteration, the dose and location of injection was controlled (that is, an animal dosed with 0.55 ml of ketamine–acepromazine intramuscularly in the left thigh for nonjacketed plethysmography was given the same dose in the same location 7 d later for plethysmography while wearing the jacket), and an attempt to control the time from injection to starting WBP was made. However, controlling times from injection to start was difficult, as the response to anesthesia varied not only among animals but also within individual animals from week to week. During the first iteration, 1 animal

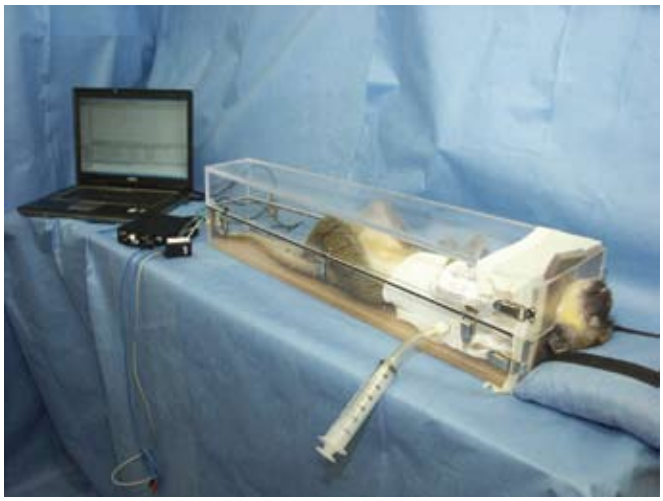

Figure 1. African green monkey fitted with a nonrestraining protective jacket and positioned in a medium plethysmography box.

Table 1. Iteration 1 results (n = 12)

	Variable	Mean ± 1 SD	*P*
Accumulated volume (ml)	No jacket	911 ± 318	0.0510
	Jacket	720 ± 256	
Minute volume (ml)	No jacket	593 ± 188	0.0604
	Jacket	486 ± 141	
Respiratory rate (breaths/min)	No jacket	32 ± 10	0.1688
	Jacket	28 ± 10	
Tidal volume (ml)	No jacket	20 ± 5	0.3841
	Jacket	18 ± 5	

Table 2. Iteration 2 results (n = 12)

	Variable	Mean ± SD	*P*
Accumulated volume (ml)	No jacket	1137 ± 357	0.6824
	Jacket	1190 ± 365	
Minute volume (ml)	No jacket	749 ± 193	0.7229
	Jacket	775 ± 217	
Respiratory rate (breaths/min)	No jacket	29 ± 7	0.1116
	Jacket	25 ± 7	
Tidal volume (ml)	No jacket	29 ± 13	0.1556
	Jacket	33 ± 10	

required additional anesthesia (1/3 of the original dose) to reach an anesthetic plane that would allow placement of the jacket, positioning of the animal within the box, and collection of respiratory data for 3 min. Although use of a different anesthetic regimen (that is, inhalant anesthesia or constant rate infusion) may have allowed greater standardization of anesthetic depth,[4] we chose the ketamine–acepromazine combination because it is the standard anesthetic regimen used for plethysmography and aerosol biothreat exposures in African green monkeys in our facility. The difficulty in controlling the plane of anesthesia is not unique to our laboratory—similar difficulty in maintaining precise levels of anesthetic depth for plethysmography has been reported recently.[11] The use of an inhalant anesthetic regimen would interfere with the experimental endpoint because nonhuman primates cannot be exposed to an aerosol while breathing through an anesthesia circuit. In addition, the physical limitations within the class III biological safety cabinet, along with the propensity for the commonly used continuous intravenous infusion anesthetic agents to cause respiratory depression, preclude their use in a constant-rate infusion regimen with this model. Although tiletamine–zolazepam might be considered as an alternative intramuscular anesthetic for use during aerosol exposures, our institute has, anecdotally, experienced adverse events when using this drug in green monkeys and thus avoid its use in this species.

Our data show that WBP can be performed on African green monkeys wearing protective jackets, with no significant alterations in minute volume, tidal volume, respiratory rate, or accumulated volume. These findings allow the jackets to remain in place on all nonhuman primates during the plethysmography–aerosol-exposure procedure, with confidence that calculations for inhaled dose are not being compromised.

Acknowledgments

The views, opinions, and findings contained herein are those of the authors and should not be construed as official Department of Army positions, policies, or decisions unless so designated by other documentation. The authors do not have commercial or other associations that would pose a conflict of interest. We are grateful to Katheryn F Kenyon, Alec S Hail, Christopher S Gamble, and Norman Kresielmeier for critically reviewing this manuscript and to Eugene Blue and Jamie Blue for their technical assistance.

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