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Research article

Evaluation of a method utilizing PhysioFlow®, a novel signal morphology-based form of impedance cardiography, to measure cardiac output in the conscious beagle

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ABSTRACT

Introduction: Currently, standard methods for measuring cardiac output are either invasive (i.e. flow probe) or are limited in terms of short measurement intervals and measurement variability (i.e. echocardiography). The ability to reliably measure cardiac output in a non-invasive manner in large animals would provide a valuable tool to expand functional cardiovascular endpoints in preclinical safety studies. PhysioFlow® is a novel method that uses waveform analysis of an impedance signal to measure cardiac output non-invasively. Unlike cardiac impedance techniques in the past, PhysioFlow® is not dependant on thoracic structure or basal thoracic impedance (Z_0) and therefore this methodology is transferrable from human to animal models.
Methods: Three tool compounds with known effects on cardiac output were administered to conscious beagle dogs to determine if the non-invasive PhysioFlow® system could detect the expected changes in stroke volume and cardiac output as determined by literature references using the current standard methodologies (e.g. aortic blood flow and thermodilution).
Results: The PhysioFlow® system was able to detect increases in cardiac output when dosed with 20 μg/kg of Dobutamine, a decrease in cardiac output when dosed with 0.1 mg/kg of Acepromazine, and no significant change in cardiac output when dosed with 2 mg/kg of Minoxidil. These results are within expected ranges based on published literature (Stepien et al., 1995; Taylor et al., 2007).
Discussion: PhysioFlow®, a signal morphology-based impedance cardiography, can be utilized to reliably and non-invasively measure cardiac output in beagle dogs.

1. Introduction

Preclinical safety pharmacology studies assessing the effects of new chemical entities on cardiovascular function in large animal models using telemetry often detect alterations of arterial blood pressure. Identifying whether these alterations are resulting from a change in cardiac output or vascular resistance can be useful information to the discovery teams when trying to understand mechanism of action and the safety implications of drug targets. Direct methods of measuring cardiac output, such as flow probes and thermodilution are invasive, expensive, or require specialized technical skills. The current standard for measuring cardiac output using thoracic impedance is a volumetric approach to determine basal thoracic impedance (Z_0) and the pulsatile variation in impedance (ΔZ) (Charloux et al., 2000). Some of the limitations of this volumetric approach to cardiac output measurements include the need to take into account size and shape of the chest, body fat, thoracic fluid and placement of electrodes (Bour & Kellet, 2008). PhysioFlow® is a novel technology that measures stroke volume using impedance waveform morphology analysis and a high performance noise cancelation filter that removes the need to use basal thoracic impedance (Z_0), allowing for non-invasive measurements in a variety of different species with less limitations. The PhysioFlow® system has been approved by the FDA for measurement of stroke volume and cardiac output in humans. The ability to utilize PhysioFlow® as a method of non-invasively measuring stroke volume would be beneficial as a supplemental endpoint in preclinical studies that allows for the addition of cardiac output and peripheral vascular resistance measurements.

The primary non-invasive method currently used to measure cardiac output in non-rodent cardiovascular safety pharmacology studies is echocardiology. However, this technique involves specialized training and its methods for volumetric calculations can produce variable results. There are other direct techniques available to measure cardiac output using implantable devices such as aortic flow probes. However, these methods have a variety of limitations including their invasive nature requiring surgical implantation or anesthetized preparations. The ability to reliably measure cardiac output in a non-invasive manner in conscious large animals would provide a valuable tool to assist in the characterization of drug effects on cardiac and vascular functions in preclinical safety studies.

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In the current study, compounds with known effects on cardiovascular function were used to determine if the PhysioFlow® system is capable of detecting changes in stroke volume and cardiac output with the accuracy and precision of other methods. A single bolus dose of acepromazine was administered intravenously to determine if the system was able to detect the expected decreases in stroke volume and cardiac output, while a series of escalating doses of dobutamine were administered intravenously to determine if the system could detect the expected increases in stroke volume and cardiac output. Finally, a single oral dose of minoxidil was administered to determine if the system was able to detect the expected decrease in stroke volume and increase in cardiac output.

2. Materials and methods

2.1. Statement on use and care of animals

This study was performed with the approval and oversight of the animal care and use committee. All procedures were performed as per the standard operating procedures (SOPs) and animal use protocols (AUPs) and conducted in accordance to the principles set forth in the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011). GlaxoSmithKline US is an AAALAC accredited institution.

2.2. Animals and maintenance

Four male purebred beagle dogs obtained from Marshall Farms USA, Inc. (North Rose, NY) were used in this study. Dogs were approximately 1 to 10 years of age and weighed between 9 and 12 kg at the initiation of dosing. Each dog was identified by a unique number tattooed on the inside of the ear. All dogs were housed individually in stainless-steel cages and were acclimated to local housing conditions for at least seven days prior to the initiation of dosing.

Telemetry units, with 2 pressure sensitive catheters, 2 biopotential (ECG) leads and a body temperature probe (Model No. TL11M3-D70-PCTP, Data Sciences International (DSI), St. Paul, MN) were surgically implanted prior to the animals being selected for use in this study. The surgical implantation of the telemetry devices involved performing a thoracotomy using sterile technique under general anesthesia. The body of the transmitter (including the thermistor) was placed between the muscle layers in the left flank. For the evaluation of cardiovascular parameters, a pressure sensitive catheter was inserted into a femoral artery as well as the left ventricle of the heart. For the collection of the electrocardiogram, the ECG electrodes were tunneled to the thoracic cavity and, following a thoracotomy under positive pressure ventilation, sutured to the pericardial surface at the base and apex of the heart in order to create a bipolar ECG lead across the heart (base-apex lead). Telemetry was used only to measure arterial blood pressures, cardiac pressures and ECG parameters were not collected in this study.

The environmental controls were set to maintain temperature within the range 64 to 84 °F and relative humidity within the range 30 to 70%, with an approximate 12-hour light/dark cycle.

A daily allotment of approximately 300 g of LabDiet™ brand Certified Laboratory Canine Diet #5007 (PMI™ Nutrition International, Richmond, IN, USA) was offered daily. Filtered tap water (supplied by Aqua Pennsylvania, Inc. and periodically analyzed) was available ad libitum from an automatic watering source.

Available information on the diet used and routine periodic analysis of the water in this facility does not indicate the presence of any substance at a concentration likely to influence the outcome of this study.

A general check including availability of food, water and gross environmental conditions was made each day of the study.

2.3. Body weights and acclimation

Body weights of all test animals were obtained for dose calculations prior to each dose on the day of dosing. To reduce handling-related stress on the days of measurements involving restraint, all dogs were acclimated to restraint procedures on at least 11 occasions for periods of up to 75 min. To reduce stress related to dosing, dogs were acclimated to oral gavage dosing with water on 2 occasions prior to study.

2.4. Study design

Daily random assignment to the dosing sequence for each treatment was ranked by body weight using The SAS™ System for Windows™ (Release 9.1, SAS Institute, Cary, NC) random sequence command PROC PLAN. Body weights obtained prior to each dose were used to calculate doses. Dogs were placed in slings and both telemetry and PhysioFlow® data was collected for approximately 20 minutes prior to dosing. PhysioFlow® and telemetry data was collected as 1 min means during the course of all collections. Minimum washout time between each treatment was 7 days.

For treatment 1, each of the 4 dogs received a single bolus dose of acepromazine (0.1 mg/kg) delivered intravenously, after which telemetry and PhysioFlow® data continued to collect for approximately 20 minutes post-dose. After this 20 minute collection dogs were returned to their home cage. Pre-dose means were calculated using the 20 min prior to dosing. The post-dose means were calculated using the final 10 min of collection post-dose, allowing for the animal to reach a steady physiological state after dosing of acepromazine.

For treatment 2, each of the 4 dogs received a slow saline infusion to maintain catheter patency during baseline measurements followed by three consecutive 20 minute infusions of dobutamine (5, 10 and 20 μg/kg/min). Telemetry and PhysioFlow® data was collected until the end of the final infusion. Once the final infusion was complete the dogs were returned to their home cage. Pre-dose means were calculated using the 20 min prior to start of dobutamine infusion. The post-dose means were calculated using the final 10 min of each dobutamine infusion, allowing for the animal to reach a steady physiological state after each dose of dobutamine.

For treatment 3, each of the 4 dogs received a single oral dose of minoxidil (2 mg/kg). After dosing the dogs were returned to their home cage. Based upon the timing of C_{max} from previous literature (Taylor, Patel, & Sullivan, 2007), the dogs were put back into the sling and approximately 20 min of telemetry and PhysioFlow® data was collected 2 h after dosing. Once this collection was complete the dogs were returned to their home cage.

2.5. Experimental procedures

2.5.1. Animal preparation

Conscious dogs were placed into slings for approximately 20 min prior to dosing. To ensure acclimation to the sling, each dog was placed into a sling for up to 75 min on at least 11 occasions prior to the treatment period. The neck and chest of the dog were shaved with clippers and cleaned with alcohol to allow for good contact of electrodes to the skin. Six electrodes were placed on the dog to allow for cardiac impedance measurements to be collected. Two electrodes are placed on the left lateral aspect of the neck (Z1 and Z2), 1 electrode was placed on the right midclavicular line near the 3rd intercostal space (EKG1), 1 electrode was placed on the left midclavicular line near the 7th intercostal space (EKG2), and 2 electrodes on and just below the xiphoid process of the sternum (Z3 and Z4). Vet wrap was then used to help secure the electrodes and help maintain a good signal. Electrodes were then attached to the PhysioFlow® acquisition system (version 1.0.7 RC9.15 SVV Edition Lab1 Enduro, MANATEC BIOMEDICAL, Bristol, PA).