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Original article

Background variability in standard clinical pathology biomarkers in beagle dogs instrumented with chronic indwelling telemetry devices

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ABSTRACT

Introduction: Contemporary best practice recommendations in preclinical cardiovascular safety assessment promote 3Rs principles. This includes the employment of within-subjects experimental designs to evaluate discrete, acute doses of investigational new drugs, as well as the maintenance of stock colonies of appropriate large animal test systems. Such colony species are often tested repeatedly on independent studies with provision of appropriate recovery periods and requisite health status evaluations (e.g., physical examinations, electrocardiographic assessments, clinical pathology evaluations). **Methods:** To investigate the utility of the often reiterative process of pre- or inter-study clinical pathology testing to help ascertain health status of non-naïve, telemetered canines (beagle dogs), the present study collated the results of a randomly selected set of animals approximately every three months for a period of three years. **Results:** Although occasionally a few routine hematology or clinical chemistry endpoints did demonstrate evidence of systematic trending over time, none of the observed fluctuations fell outside the range of expected biological variability, nor would have prevented assignment of any given animal to study. **Discussion:** The present findings illustrate a high degree of consistency in routinely assessed clinical pathology parameters during the course of chronic telemetry instrumentation in the canine, including relative to historical control data in healthy, experimentally naïve animals of the same species and source, maintained under analogous laboratory conditions. The data suggest that routine assessment of such parameters for the purposes of facilitating judgments concerning suitability for study may represent a pursuit of little overall value, and which may be reasonably accomplished based on alternative, observation-based screening procedures.

1. Introduction

Hematological and clinical chemistry parameters have been utilized historically in toxicology testing to inform on functional changes of significance, which often may be meaningfully correlated with organ/tissue histopathological findings (Auletta, 2002; Denny & Stewart, 2013). Among the data collected during the course of drug safety evaluations, clinical pathology panels are often uniquely informative of key organ functions, and may serve as biomarkers which facilitate the conduct of pre-clinical toxicology testing, and ideally, clinical trial study conduct (York, 2013). In addition to their utilization in toxicology testing, with other ancillary observations, the assessment of clinical pathology biomarkers is generally useful as a means of establishing the health status of test subjects and accordingly has been employed to exclude potentially compromised animals from evaluative and/or invasive procedures (e.g., Baird, Dalton, & Gauvin, 2014; Baird, Gauvin, & Dalton, 2013).

The use of telemetry for the purpose of monitoring fundamentally important physiological variables in basic and applied research has been proliferative, particularly over the past 10–15 years (Redfern & Valentin, 2011). As the telemetry technology designed for monitoring cardiovascular function has evolved concomitantly with regulatory guidance (Anonymous, 2001, 2005) and industry perceptions of best practices (Cavero, 2010; Leishman et al., 2012), it has become common for testing facilities to maintain dedicated colonies of chronically implanted telemetry animals from which to draw repeatedly for the purpose of assessment of cardiovascular safety (Baird, Gauvin, et al., 2013). An important consideration in this practice, so as to not to introduce the possibility for experimental confound related to compromised health status, is the application of appropriate procedures that may inform on the health status of any individual animal as part of the assessment of eligibility for study. To this end, detailed clinical and veterinary physical observations, electrocardiograms, and selected panels of clinical pathology endpoints have been employed in many laboratories (Baird et al., 2014). The apparent utility of such measures seems relatively obvious to the goal of screening for healthy test subjects, particularly prior to exposure of such subjects to invasive surgical procedures. It is clearly the case that the performance of telemetric evaluations to confirm cardiovascular status is valuable in a variety of circumstances (Baird, Gauvin, et al., 2013; Baird et al., 2014; Chui, Derakhchan, & Vargas, 2012; Cools et al., 2011; Guth et al., 2009;

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Leishman et al., 2012). However, the general utility of periodic and/or repeated clinical pathology screening has not been validated in data-based, longitudinal examination of telemetry colonies in routinely utilized species. Indeed, the application of routine clinical pathology screening may be of questionable value in consideration of the recent reports indicating no expectation of a change in routinely assessed parameters when general facility animal care programs, and specifically peri-operative care in support of telemetry, are optimal (Baird, Bailie, et al., 2013; Baird, Gauvin, et al., 2013; Mitchell et al., 2013; Walisser et al., 2013).

This investigation accordingly was conducted in a large sample of chronically telemetered beagle dogs to determine the nature of essential variation in a standard panel of hematology and clinical chemistry biomarkers both prior to surgical implantation and for up to three years post-implantation. The goal of the experiment was to identify trends in these selected parameters over time, particularly those which might be attributable to surgical procedures and recovery and/or in vivo persistence of the telemetry device and to view these data in relation to other routine pre-study qualifications to facilitate a possible consensus view on the relative value and corresponding need for such pre-study measures.

2. Methods

2.1. Test system

All procedures were conducted in compliance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals within an AAALAC-accredited test facility. The animals were received from a USDA-approved laboratory animal vendor previously audited (including requisite site inspections, as required) by staff veterinarian members of the MPI Research IACUC. Seventy-three (N = 73; 53 male, 20 female) beagle dogs (Covance Research Products, Kalamazoo, MI), 5–6 months of age, surgically instrumented with telemetry transmitters, were identified from the test facility telemetry stock colony and evaluated periodically (approximately every three months) for a number of standard hematology and clinical chemistry parameters over a three-year period post-surgical implantation.

On average, telemetered beagle dogs selected for study were ultimately placed on 5 ± 2 (mean ± SD) different studies (average inter-study interval of 118 ± 36 days, with an average of 113 ± 107 days prior to initial study assignment [mean ± SD]). All studies involved the safety evaluation of small molecules of various therapeutic classes, and all animals were allowed a washout/recovery period of at least 4 weeks prior to blood sampling for clinical pathology determinations.

2.2. Telemetry implants and surgical procedures

The animals were surgically instrumented with fully implantable Data Sciences International (DSI) TL11 M2 D70-PCT telemetry devices. The specific surgical procedures have been described in detail elsewhere (Baird, Bailie, et al., 2013; Baird, Gauvin, et al., 2013; Holdsworth et al., 2013), but briefly, the DSI transmitter body was secured within the abdominal cavity, with arterial pressure catheter placed into the internal iliac artery and advanced into the descending aorta, and ECG leads fixed in an epicardial arrangement within the thoracic cavity. Appropriate peri-operative medications were administered, and pain control regimens followed for each animal, with at least a two-week recovery period prior to placement of any animal on study.

2.3. Clinical pathology evaluations

Hematology and serum biochemical evaluations were periodically performed to determine the long-term effects of device implantation on routine clinical pathology endpoints (Tables 1, 2). Hematology assessments were conducted on Advia 120 or 2120 (Siemens, Munich, Germany) hematology instruments using EDTA anticoagulated whole blood and included leukocyte count, erythrocyte count, hemoglobin concentration, hematocrit percentage, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelet count, reticulocyte count, and absolute counts for neutrophils, lymphocytes, monocytes, eosinophils, basophils, and large unstained cells. Serum biochemical analyses were performed on the Olympus AU2700 or 640 (Beckman Coulter, Brea, CA) clinical chemistry instruments and included measurement of the concentrations of sodium, potassium, chloride, urea nitrogen, creatinine, total protein, and glucose. For any individual animal, at least a four week interval was instituted between the time of completion of study and prior to the next clinical pathology sampling to avoid potential preanalytical variation due to study manipulation or procedures. Data are summarized quarterly (e.g., every three months) and presented with historical control reference ranges.

2.4. Data analyses

Since historical control data and preliminary data analyses revealed no demonstrable gender-based differences in measured clinical pathology endpoints, data from male and female animals were pooled for presentation. Study data generated from the selected sample of beagle dogs were evaluated in relation to samples of testing facility historical control data obtained from beagle dogs from the same vendor, encompassing a similar, though slightly truncated range (0.5–1.5 years). Specifically, Tables 1 and 2 contain a mean ± SD reference value, and Figs. 1 and 2 contain an indication of the 95% confidence interval, calculated from these historical control data.

Summary and individual animal data were evaluated with respect to the extent of deviation from historical control data; in addition, ancillary data records collected at the same time period were evaluated for the presence of any additional, potentially corroborative findings indicating a departure in health status, including clinical veterinary and/or other observations.

3. Results

Figs. 1 and 2 depict summary data for hematology and clinical chemistry parameters, respectively, evaluated prior to surgical telemetry device implantation (time 0) and for three years subsequently. No discernable effects on either individual or summarized hematology or clinical chemistry parameters were identified at any point throughout the evaluation period. All fluctuations were considered within an acceptable range for expected biological variation in experimentally naïve animals (e.g., within 2 standard deviations of the historical control or pre-study mean, with no corroborative observational indications of departure from nominal health status). Although periodic, minimal departures from historical control values derived from surgically naïve animals (shaded regions = 95% CI) were observed on a limited basis for summarized data, and somewhat more frequently in individual animals, none of these deviations, including in individual animals, were considered meaningful based on their overall magnitude, direction, and/or pattern over time, nor were these correlated with any corroborative clinical veterinary or other observations to suggest a condition contraindicating viability of any animal for assignment to study.

Several trends among hematology values were noted that were consistent with known age-related effects typically expressed as juvenile canines' transition to adulthood. Leukocyte concentration demonstrated a mild decrease over time that was mostly attributable to reductions in lymphocyte and monocyte counts. Reductions in leukocytes in growing canines have been described previously and are even more pronounced in animals less than 3 months old tracked through adulthood. (Harper, Hackett, Wilkinson, & Heaton, 2003) Among serum biochemical analytes, a mild upward trending of total protein concentration was