Evaluation of Shipping Stress in Surgically Altered Rodent Models

Lauren Lubejko[1], Steven Kreuser[1], Jason S. Cordes[2], Venkateswarlu Karicheti[3], Jan Bernal[1], Cedo M. Bagi[1]

[1]Surgery and Anesthesia, Global Science and Technology, Comparative Medicine, [2]Drug Safety Research and Development, Pfizer Inc., Groton, CT 06340, [3]Surgical Services, Charles River Laboratories, Raleigh, NC 27610

Introduction

Pfizer currently outsources the production of many rodent surgical models to vendors across the country for delivery to Pfizer locations. At this time, the only approved method for shipment is by ground courier due to concerns about the impact of shipping stress on the animals. Though every effort is made to limit the animal's transit time from vendor to Pfizer, it can sometimes take up to four days from origin to destination. Given there is limited published information on the duration of physiological stress indicators during the time before, during, and after transportation, the objective of this study was to evaluate stress in rodents following surgery in relation to time of shipment and acclimation, and to assist in establishing guidelines for humane shipping post operatively. We focused our investigation to 24 hours prior to shipment and 72 hours post-delivery.

Methods

All procedures involving animals were in accordance with regulations and established guidelines and were reviewed and approved by Pfizer's Institutional Animal Care and Use Committee. Starr_Oddi DST micro-HRT data logger devices were purchased to evaluate heart rate and temperature in rodents from the time of surgery to delivery and acclimation. After programming the devices, they were double bagged, gas sterilized and shipped to Charles River Laboratories* (CRL), Raleigh, NC. Upon receipt, the data loggers were delivered to the surgery suite, removed from packaging and placed in cold sterilant over night to ensure sterilization. The devices were rinsed aseptically before implanting in animals.

Twelve male, 8-10 week, variable weight, CRL CD Sprague Dawley rats were used. Group A (n=6) were rats that had control data logger implants only. Group B (n=6) had both jugular catheter and data logger implantation. Surgery took place on day 0.

Surgical Description: (Provided by CRL)

Group A: The rats were anesthetized with ketamine (75 mg/kg) and xylazine (6.0 mg/kg) administered intraperitoneally and provided buprenorphine (0.02 mg/kg) subcutaneously. The skin overlying the right jugular vein is shaved and aseptically prepared using chlorhexidine and alcohol. A 1.0 to 1.5 cm cranial-caudal incision was made to expose the right jugular vein. The data logger was subcutaneously tunneled to place on the left chest (close to apex of the heart). The skin incision was closed using subcuticular suture.

Group B: The jugular vein was isolated and ligatures were placed using non-absorbable suture material. A small incision was made in the jugular vein and a polyurethane catheter was inserted into the vein, and a ligature was subsequently tied around the cannulated vessel to fix the catheter in place. The extravascular portion of the catheter was tunneled subcutaneously to the dorsal scapular region. The catheter was locked with heparinized dextrose solution. The distal end of the catheter was heat sealed. The skin incision was closed using subcuticular suture.

Methods (cont.)

Immediately following surgery, daily clinical and behavior assessments were completed as per CRL guidelines. The behavioral observations were recorded which evaluates movement, posture, body condition, respirations, and other parameters. On day 3, a physical exam was performed including body weight assessment. The rats were then packaged, and shipped from CRL, Raleigh, NC, to Pfizer, Inc. Groton, CT. During shipping, any major events such as on-road inspections transfer to holding areas, and other significant events were logged. On day 4 the rats were delivered to the Groton site where they were unpacked, had a physical exam, body weight assessment, and were placed in home cages. Daily log entry was completed to record significant events as well as behavioral observations within the assigned housing room. Body weights were collected at the time of surgery, prior to shipment, and receipt in Groton and on days 7 and 14. On day 14 rats were observed, assessed for body weight, euthanized, and data loggers were collected. Post day 15 the data was retrieved and analyzed.

Discussion

Understanding shipping stress of surgically altered rodents is relevant to animal welfare as well as the science and research that these models support. The changes in normal physiological levels associated with shipment related stress can affect scientific validity and consequently alter study results. The focus in this study was heart rate which is one of the physiologic parameters previously documented and associated with stress. Based upon this assumption significantly increased heart rates were correlated with increased stress.

Results from this study determined that the surgical group (B) displayed statistically significant higher heart rate values than those from the non-surgical group (A) (Figure 1). Group B consistently maintained a higher heart rate value than Group A. T-test analysis showed that Group B and A were statistically different from one another, however, when comparing the two groups during pre-shipment , transit, post-transit (24, 48, and 72 hrs.), they were proportionately increased (Figure 2). The standard deviation of differences between each time frame was within a close range of points from one another (range 25.43-31.98). This data illustrates that the rats in Group B did not exhibit any evidence of additional stress due to transit, but the consistently increased heart rate can be contributed to the surgical procedure. In addition, the diurnal patterns of the rats were observed from the heart rate data with a variance in the pattern during transit but illustrated a normal pattern within 72 hours after arrival at the destination facility.

Discussion (cont.)

Additionally, body temperature was collected and analyzed as a potential indicator of stress during transit. The body temperature changes noted directly relate to the drop in ambient temperature inside of the transport truck (Figure 3).

The body temperatures from both Group A and B remained fairly constant throughout transit, and were not significantly changed throughout that time, with the correlation coefficient being 0.8. Finally, body weight measurements and behavioral observations were collected pre-shipment, transit and post-transit with no significant differences between the two groups.

Many stress evaluation studies include cortisol levels. Cortisol levels were not factored into this study because the effects of stress and the associated levels have previously been researched extensively and documented in the literature. The focus of this study was evaluation of stress associated parameters collected during the actual transportation and there was not a feasible method for collection of the required samples during shipping. Previous publications are used to guide the assessment of the data for our conclusions with respect to cortisol levels.

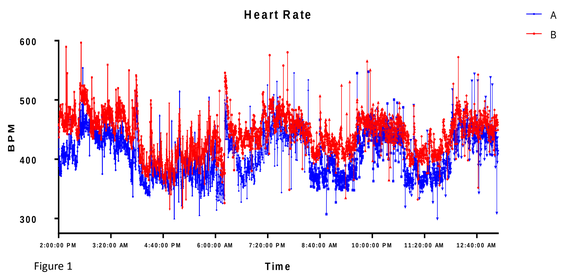

Figure 1

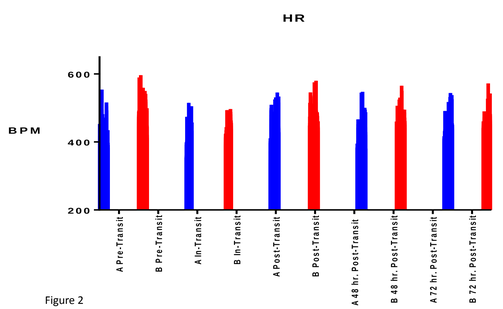

Figure 2

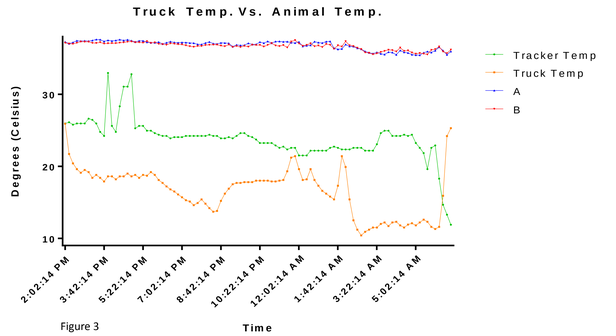

Figure 3

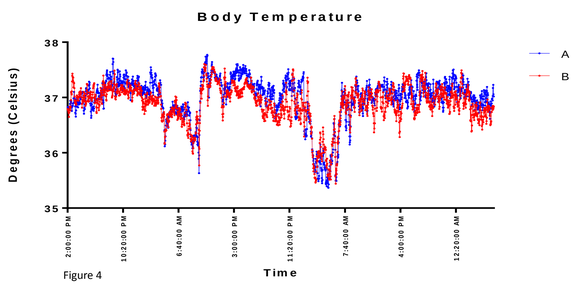

Figure 4

Conclusion

The focus of this study was to evaluate the physiological indicators of stress in surgically altered rodents during the process of shipment. During transportation, both the surgical (B) and non-surgical group (A) responded similarly with both temperature and heart rate values. The results of the changes in heart rate during the time points evaluated indicate that the surgical status of the rats did influence the heart rate, but the physiologic response to shipping was independent of the surgical status.

This study utilized a novel method to collect data during the transportation (shipment) and acclimation period which can be used as a foundation for additional studies to continue to evaluate and refine current shipping practices with regards to rodent surgical models.

Acknowledgments

The authors would like to thank Cassandra Rider, Evan Pagano, Donna Zyry and the Charles River surgical staff for their assistance with this study.

Pfizer WORLDWIDE RESEARCH & DEVELOPMENT
Comparative Medicine

SCIENCE FOR LIFE-CHANGING IMPACT