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Performance and Consistency of Circulating Warm Water Blankets for Rodents

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General anesthesia as used for rodent research can have adverse effects on physiologic mechanisms. Thermoregulation is often greatly inhibited, with resultant deleterious effects on cardiac and respiratory function. These potential effects can be mitigated by providing external heat support. The circulating warm water blanket and associated heat pump are often used in rodent procedures. The current study demonstrated that the heating pump and water blanket require quality control assessment to ensure adequate function. Our data showed that of the 6 pumps tested, 5 were able to achieve a temperature that met or exceeded the documented thermoneutral zone for mice. Pumps required 20 min of warming to reach their maximal attainable temperatures for the designated user setting. Although the pumps reached a temperature that was sufficient to provide external thermal support, only 1 of the 6 pumps reached the temperature that was set by the user during the trial. Surface temperatures across the water blanket were recorded to analyze whether a difference in heat support was influenced by animal placement along the water blanket; however, the location points did not yield statistically different results. Two pumps were eliminated from the study due to failure to pass the preparation phase of the trial. The results of this study support the need for facilities to establish quality control measures to ensure that heat support systems are functioning at a level required to maintain normothermia during anesthetic procedures.

Abbreviations: CWWB, circulating warm water blanket; TNZ, thermoneutral zone

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Introduction

General anesthesia is often used in preclinical rodent studies to support biomedical research. Rodents can be anesthetized with injectable or inhalant agents, both of which have inherent risks. In conscious animals, core body temperature is highly regulated. The autonomic nervous system combined with instinctive behaviors of the animal serve as mechanisms of temperature regulation. Thermoneutrality is the ambient temperature at which the metabolic rate is at a minimum.[14] Typical housing temperatures for research mice are below their thermoneutral zone (TNZ), which has prompted research into thermal stress.[7,14] Thermal stress can have a significant impact on study outcomes and data variability.[7] The TNZ of mice has a particularly narrow range of 29.6 to 34 °C (85.2-93.2°F).[8] Small changes in the ambient temperature range can have profound effects on neuronal function and the circulatory system. Afferent inputs from temperature receptors throughout the body, following various neuronal pathways, ultimately arrive at the thermoregulatory center in the hypothalamus triggering an autonomic response.[3] The hypothalamic threshold range is defined as the temperature range that does not trigger a thermoregulatory autonomic response.[3] General anesthetics can depress hypothalamic responses, resulting in widening of the threshold range and triggering a redistribution of blood from central to peripheral vasculature and an overall loss of heat from the body to the environment.[3,11] An inability to efficiently thermoregulate leaves animals vulnerable to hypothermia, which can have deleterious effects on cardiac function.[11] In addition, hypothermia poses an anesthetic toxicity risk due to the body's decreased ability to redistribute and eliminate pharmacological agents.[3] Maintaining normothermia during anesthesia is particularly important in small mammals because their high surface area to body weight ratio makes them particularly vulnerable to changes in temperature.[2,4,5,6,8,16] Because of the effect of general anesthesia on the hypothalamus, hypothermia develops before thermoregulatory mechanisms, like vasoconstriction, are triggered.[11] Once established, hypothermia is difficult to manage.[11] Prewarming can delay the onset of hypothermia after anesthesia induction by reducing the temperature gradient between core and peripheral tissues after the redistribution of blood in response to temperature-dependent physiologic vasoconstriction.[11,12] Providing supplemental thermal support during anesthesia is the primary method of preventing hypothermia.[16,17]

Several technologies have been developed to provide external heat support. Two that are often cited in literature are the circulating warm water blanket (CWWB) with an associated temperature-controlled heating system and the forced air system.[13,17] Rodents are the most common animals used in biomedical research, and the CWWB is cited as one of the technologies most often used in rodent procedures.[16] Several studies document the benefits of using external heat support during anesthesia. One study in rats demonstrated that forced-air prewarming prevented hypothermia during anesthesia by increasing core body temperatures.[11] A study that evaluated the effectiveness of different thermoregulatory devices during anesthesia in mice found that the CWWB on a medium temperature setting was the most consistent method of maintaining temperature over time between mice.[2] The use of external heat

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therapy systems in rodents and comparisons between various device modalities is well documented.[1,2,11] However, less peer-reviewed literature is available that specifically documents the potential for variability between individual devices of the same type and provides standard methods for ensuring quality control.[17] The efficacy of external heat sources is directly dependent on the accuracy and consistency of the device; if thermal support is too hot or too cold, it can have confounding effects on the study and potentially detrimental or fatal effects on the anesthetized rodent.

The purpose of this study was to conduct a controlled assessment of a heat therapy system with an associated CWWB in order to establish a standard operating procedure to assure that this equipment would provide effective and consistent thermal support to laboratory rodents in our facility. We hypothesized that the surface temperature of the CWWB connected to the temperature-controlled heat pumps would maintain a physiologically appropriate temperature for a rat or mouse independent of the time the pump was activated or its location within the CWWB. The results of this study support the need for facilities to establish quality control measures to ensure that heat support systems are functioning at a level required to maintain normothermia during anesthetic procedures. These quality control measures can assure adequate thermal support, reaching and maintaining a selected appropriate temperature to decrease the risk of hypothermia secondary to anesthesia or hyperthermic burn injuries.

Materials and Methods

Equipment and pump preparation. Six Gaymar T/P-500 (Gaymar Industries Orchard Park, NY) circulating hot water pumps were used for this experiment. A single, new foldable MuloToPad water blanket (Braintree Scientific, Braintree, MA) was used for each pump trial and was connected to the pump using clik-tite connectors that are included with the Gaymar heat pump and the MuloToPad water blanket. All trials were conducted by the same operator, in the same room, with temperature and humidity kept within a target range. The criteria for exclusion of units were based on the model of the unit, its documented age, the frequency of use in the facility, and the unit's ability to successfully complete the preparation phase of the trial. The units used were housed in rooms that had a comparable frequency of operations, were of the same model number, and were of the same age (Gaymar T/P Classic, Model # TP-500, 120 V, 6.0 Hz, 200 W, 1.8A, P/N 07999-000, 5/95). Before initiation of the experimental phase, the pumps were prepared for use based on manufacturer recommendations. The external surface was cleaned using a damp cloth and Clidox-S (Pharmacal Research Laboratories, Waterbury, CT), which is the standard cleaning solution for the facility. The pumps were disconnected from the CWWB and drained of water. The reservoirs were then filled with distilled water and 1/4 ounce of Clidox-S to the operating level indicated on the side of the pump. For the pump preparation phase, the temperature was set to the manufacturer's recommendation at 30 °C (86° F) and the pump was turned on for a 1-h circulation cycle. After one hour of cleaning, the pumps were prepared for the experimental phase; the cleaning solution was disposed of, and the pumps were filled with distilled water to the operating level indicated by the fill line. The pumps were then connected to the CWWB and turned on. Additional distilled water was added to keep the water level within the system at the operating level indicated by the fill line prior to turning off the pump before initiation of the experiment.

Experimental methods. All assessments were conducted over a 3-d period in a single room in Tufts University (Boston, MA) rodent facility. The pumps were tested in random order. The ambient temperature and humidity were recorded before the beginning of each experimental phase and were consistently 25 to 26 °C (77-78 °F) with a humidity of 35%. During each assessment, the pumps were connected to the new CWWB. The blanket was folded into a 7.5 × 10.5-inch configuration, allowing appropriate water flow between the pump and the blanket. The CWWB was labeled A, B, and C to indicate each measurable location point at the approximate level of the head, torso, and abdomen of a rat based on a similar study on the heating pad performance and efficacy of a CWWB using isoflurane anesthetized Sprague–Dawley rats.[20] Each point was 2-inches apart. A calibrated LaserGrip1022 Infrared Thermometer (Etekcity, Anaheim, CA) was used to measure the surface temperature of the CWWB (in degrees Celsius). As recommended by the manufacturer, a beaker stand with a clamp was used to keep the infrared gun 12 inches from the surface being evaluated, thus measuring the average temperature of a 1-inch diameter surface area. Each pump temperature was set at 42 °C (107.6 °F). for the experimental phase (Figure 1).

Temperature measurements. Surface temperatures were measured beginning immediately after the pump was turned on (time 0) and again every 10 min for a total of 60 min. At each time point, infrared temperature measurements were taken 3 times for each location (A-C). The mean temperature of each location was used for the statistical analysis; temperatures are presented as mean ± SD. Between each pump experiment, the CWWB was disconnected for 30-min to allow the blanket to return to room temperature. To ensure this had occurred, the infrared gun was then used to measure the CWWB surface temperature before beginning subsequent studies.

Statistical analysis. A 3-way analysis of variance (ANOVA) using the 3 factors of time, CWWB surface location, and pump, and the variable as the mean of the 3 measured temperatures, was conducted using Sigmaplot 14.0 (Systat Software, San Jose, CA).[19] The data did not meet the assumption of normality based on the Shapiro-Wilk normality test ($P < 0.05$), whereas the Brown-Forsythe equal variance test passed ($P = 1.00$). Because the factors time and pump showed significant differences ($P < 0.001$), a post hoc analysis was performed using the Holm Sidak multiple comparisons test. For all analyses, an α of $P \leq 0.05$ was considered statistically significant.

Results

Of the 3 factors evaluated (time, location A, B, or C on the blanket, and pump), only pump ($P < 0.001$) and time ($P < 0.001$) showed significant differences between individual pumps. No difference in location ($P = 0.214$) was detected between pumps and the connected CWWB. Time showed a significant difference between time 0 and all subsequent time points (10 to 60 min; $P < 0.001$).

Time showed a significant difference between time 0 and all subsequent time points (10 to 60 min; $P < 0.001$). A significant difference was also found between timepoint 10 min as compared with all other time points ($P < 0.001$). Comparisons made between timepoints 20, 30, 40, and 60 min did not yield any significant differences. The average time to reach the maximum recorded temperature or peak temperature for all pumps ranged from 20 to 30 min. The time to reach a physiologically appropriate temperature for a mouse (37 to 38 °C) (98.6-100.4 °F) was achieved at time point 20, except for pump 5.[5] Pump 1 had the highest average peak temperature recorded (42.9 °C ± 0.02) at

Figure 1. Photo depicts circulating warm water blanket and pump set up. Locations A, B and C are listed on the water blanket. Infrared gun and holding apparatus depicted adjacent to the blanket.

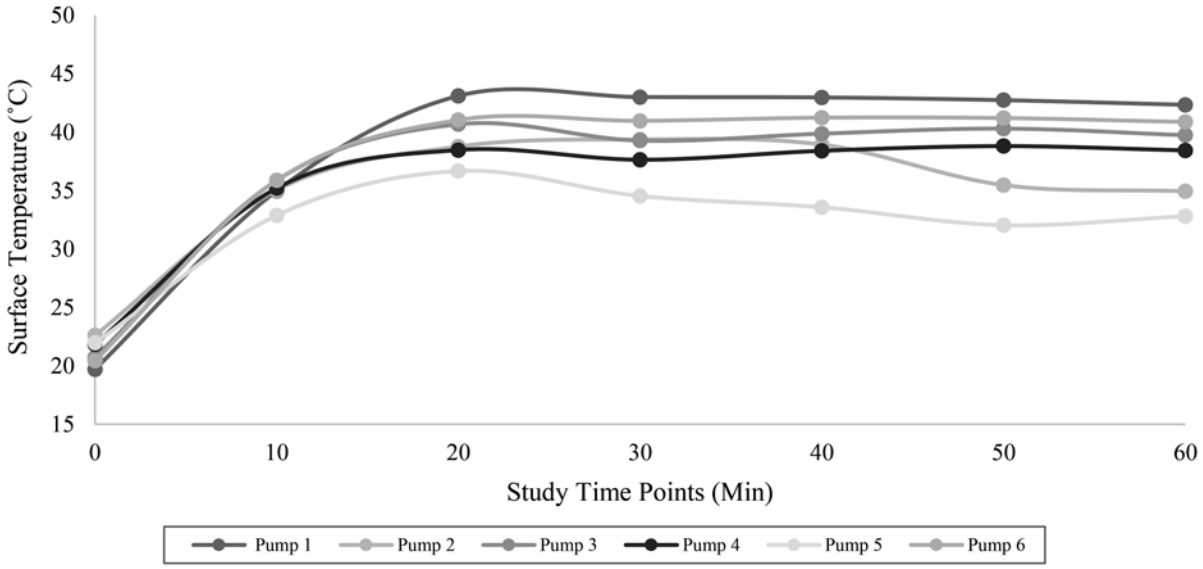

Figure 2. Average temperature at Location B for Pumps 1-6. There was no significant difference noted between location points. The average time to reach the peak temperature for all pumps ranged from 20 to 30 min. All pumps, except for Pump 5, reached a physiologically appropriate temperature for a mouse (37 to 38 °C) (98.6-100.4 °F). This supports the concept of prewarming the heat therapy system prior to use in anesthetized procedures resulting in the potential to delay or eliminate the onset of hypothermia.

time point 30 (Figure 2). Pump 5 had the lowest average peak temperature recorded (34.7 °C ± 1.7) at time point 20. No significant differences were found between the surface temperatures across locations A, B, and C for any of the pumps tested.

During the preparation phase of the study, 2 machines were eliminated from the trial because they did not warm up the heating pad despite circulating water. Manufacturer recommendations for machine repair were conducted, but the machines still did not return to the appropriate function.

Discussion

Rodents, especially mice, are some of the most studied animals in biomedical research. Physiologic factors that could have confounding effects on study outcomes must be considered when designing experiments. Although mice have relatively stable physiologic regulation, they do present unique challenges when specifically considering thermoregulation. Many of these challenges are directly affected by intrinsic factors and include their high metabolic activity, the variability of their core temperature, and their large surface area per gram of body weight.[4] Many biochemical reactions analyzed during translational research are affected by temperature, so the ability to provide adequate thermal support has a direct impact on the accuracy of study outcomes.[9] Mice have a body temperature ranging from 37.0 to 37.2 °C (98.6-98.9 °F) and a TNZ of approximately 29.6 to 34 °C (85.2-93.2 °F).[8,18] This TNZ is narrower than that of other mammals.[9,18] For this reason, providing adequate thermal support would decrease the energy required to maintain a normal body temperature.

The purpose of this study was to assess the reliability and effectiveness of commonly used external heating systems, specifically a circulating water pump and an associated heating blanket. The Gaymar T/P-500 pump is a commonly used model and is most prevalent in our facility. Having a method of assessing the pump's ability to provide adequate thermal support is valuable information. One of the principal findings of this study was the significant temperature difference between time point 10 and time points 20 to 60. The pumps, which were all set to 42 °C (107.6°F), reached a maximal temperature ranging from 38.3 to 42.9 °C (100.9-109.2°F) beginning at time point 20. Although the pumps were set to the highest available temperature, only the CWWB connected to Pump 1 reached the set temperature over the 60-min experimental duration. Pump 1 reached a temperature of 42.9 °C ± 0.02 (109.2°F), which exceeded the set temperature of the heating device. A human study found that irreversible skin damage occurred after approximately 6 h of consistent exposure to 44 °C (111.2°F).[15] In a study of the safety and effectiveness of the CWWB and the microwaveable heat pad in rats, thermal skin injury occurred as a function of temperature and contact time.[17] Thermal injury and hyperthermia are important risk factors in anesthetized animals, which have limited ability to regulate their core body temperature. Previous studies analyzing the efficacy of heat therapy systems often set their experimental temperatures at a range of 37 to 38 °C (98.6-100.4 °F), which is consistent with the normal temperature range of a mouse.[17] A temperature difference was recorded between the set temperature and the temperature provided to the animal, which is concerning if an operator is trying to target a specific temperature that is suggested for mouse anesthesia. Turning pumps on for at least 20 min allowed the mean temperature to reach 39.2 °C (102.5 °F). Although the pumps showed a deviation between the set temperature and the temperature recorded at the blanket, they did, with the exception of pump 5, reach a temperature within or exceeding the TNZ of mice. Providing external heat support that can facilitate normothermia in mice would decrease the high metabolic requirements necessary to maintain normal body temperature and mitigate many of the detrimental effects of hypothermia that ultimately affect study outcomes. However, the risk of thermal injury and hyperthermia merits additional research.

Another finding in our study was that the positioning of an animal is equivalent anywhere within the space tested (A, B, or C location) and should not affect thermal support. A previous

study analyzing the performance and efficacy of a heating pad in anesthetized Sprague–Dawley rats found inconsistencies in the surface temperature of the heating pad.[20] However, our current study found consistent temperatures. This finding is important when analyzing the efficacy of warming in subjects that vary in size.

In research environments that use shared equipment, a clear reporting method for broken and repaired equipment is important so that broken equipment is not used for experiments posing potential harm to animals. Documented standard operating procedures should be established with detailed instructions and contact information for repair when equipment is not functioning properly. The manual for the equipment should be readily accessible so that clear instructions on how to assess the functionality of the machines are available. This current study highlighted the variability that can exist between pumps. Despite the consistencies in pump age and model, attached CWWB, and set temperature, the pumps showed significant functional differences. Quality control measures ultimately allow the identification of variability and inappropriate function of equipment. For example, during the preparation phase of our study, one of the pumps was found to be overheating. Standard reporting methods would allow notification of facility management that the pump was malfunctioning, and then the manufacture recommendations could be consulted for troubleshooting and repair to prevent hyperthermic injury to animals.

To summarize, our findings showcase the variability that can exist between pumps of the same manufacturer and model within a facility. Based on the performance of the pumps, 20 min of warming after turning on the heat therapy system resulted in the pumps reaching a temperature that was supportive of the documented TNZ for mice. Based on this study, our facility now uses a standard operating procedure of prewarming the CWWB for 20 min prior to anesthetizing animals to provide adequate thermal support for rodents. Providing adequate thermal support during anesthesia is vital due to the repressive effects of inhalants on regulatory mechanisms; this is even more relevant in animals with large surface areas, which result in greater potential heat loss.[8,9] In addition, this study found no significant difference in position when comparing the location points analyzed during the 60-min cycle. This finding showed that adequate thermal support could be achieved within the analyzed segment of the CWWB surface, independent of the size of the rodent model. Ultimately, the functionality of heat therapy systems in a facility should not be assumed. Standard quality control assessments at defined regular intervals, such as described in this study, can help to ensure equipment is functioning adequately to support the physiologic processes of animals and to reduce variability when analyzing study outcomes.

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