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Using Waterless Alcohol-based Antiseptic for Skin Preparation and Active Thermal Support in Laboratory Rats

F Claire Hankenson,[1,2,*] Joshua J Kim,[2] Thien M Le,[3] Frank R Lawrence,[3] and Jacquelyn M Del Valle[1]

Rodents are frequently used for models that require surgical procedures. At our institution, laboratory rats are increasingly preferred for investigations of neurologic disorders, cardiovascular interventions, and assessment and treatment of addictive and depressive behaviors. For these types of studies, surgical preparations of the head and neck areas are necessary for catheterization and instrumentation. Based upon our former work in laboratory mice, we sought to improve rat surgery outcomes and confirm the efficacy of a waterless alcohol-based (WAB) antiseptic for skin disinfection prior to incision. In addition, we wanted to investigate whether active warming efforts improved perioperative body temperatures for rats to aid in return to consciousness. Prior to cranial surgical incision and placement in stereotactic equipment, rats were assessed after skin preparation with WAB and after thermal interventions, including prewarming cages for 30 min before anesthesia and delivery of warmed fluid (NaCl) supplementation. Core temperatures were recorded and aerobic culture swabs collected from surgical sites at multiple time points. As previously shown in mice, bacterial counts in rats were effectively diminished by WAB agents. Assessment of intraoperative body temperature trajectories did not identify appreciable differences between control rats and rats that were exposed to prewarming or warmed fluid supplementation or both. However, heavier male rats recovered more rapidly from isoflurane anesthesia than did lighter male and female rats. Although these thermal support measures did not significantly improve anesthetic recovery times in rats, animals warmed for 30 min trended toward a faster return to righting reflex after exposure to isoflurane. These findings confirm that WAB antiseptic is an acceptable option for skin preparation in rats and suggest that continued evaluation of thermal interventions remains of interest for improved outcomes in rat surgery.

Abbreviations: F, intraperitoneal warmed fluids; MALDI–TOF, matrix-assisted laser desorption–ionization time-of-flight; PW, prewarmed cage; WAB, waterless alcohol-based; WWB, warm-water blanket

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High standards of animal welfare and quality of research outcomes are best promoted through concentrated training on surgical methods, extensive presurgical planning, and compliant execution of IACUC-approved surgery protocols.[1] The *Guide for the Care and Use of Laboratory Animals* and the American College of Laboratory Animal Medicine similarly advocate for aseptic techniques in survival surgeries performed on laboratory animal species.[1,26]

Typically, skin preparation for veterinary patients consists of hair removal and cleaning of the surgery site by multiple applications of relevant chemical agents and liquid rinses to minimize the chance for postoperative infections.[2,3,17,25,29,33] Comparative studies in human, veterinary, and laboratory animal medicine have found several types and application protocols of skin cleaning agents to be equivalent in antimicrobial efficacy.[10,12,16,22-25,29,41,46-48] Given that modern animal programs typically only permit use of laboratory rodents of defined health and pathogen status, repetitive application of agents to achieve skin antisepsis in rodents may be unnecessary and has been shown to contribute to hypothermia in laboratory mice during procedures.[6,14,21]

In human and veterinary medicine, waterless alcohol-based (WAB) antiseptics are commonly used for hand-scrubbing by the surgery team, but are not specifically used for preparation of the skin site prior to surgery.[4,7,11,20,35,36,48-50] In the public realm, WAB solutions like 'hand sanitizer' gels, which contain ethanol or isopropyl alcohol or both, evaporate spontaneously without the need for rinsing. In addition, hand sanitizer gels promptly reduce the number of detectable aerobic microorganisms although they do not provide the sustained antimicrobial activity that is required for presurgical skin preparation of medical patients.[4,13] WAB antiseptics, which contain a combination of ethanol and chlorhexidine, are as effective as typical aqueous scrub agents in reducing bacteria on the skin of laboratory mice, yet require only 2 applications (no rinse needed) and mitigate heat loss during surgical skin preparation.[13] The current study extended our previous work in mice by evaluating WAB antiseptic for surgical skin preparation in rats.[6,13,44]

Rats have long been a valuable model for behavioral neuroscience, cardiovascular, and addiction treatment investigations. Often, surgical preparations of the head and neck areas are necessary for catheterization and instrumentation. Building on literature indicating that prewarming of patients prior to anesthesia could promote thermoregulation and recovery,[5,8,28,42]

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[1]Campus Animal Resources, [2]College of Veterinary Medicine, and [3]Department of Statistics and Probability, Center for Statistical Training and Consulting, Michigan State University, East Lansing, Michigan
[*]Corresponding author. Email: fclaire@msu.edu

we compared prewarming practices - the provision of warmed cage environments before anesthesia and in the early stages of the anesthetic procedure and provision of warmed intraperitoneal fluids - in study animals. To our knowledge, this is the first evaluation of WAB agents in rat surgery. The current report describes the first use of WAB antiseptic for cranial skin preparation, in combination with presurgical thermal interventions, in rats.

Materials and Methods

Animal housing and husbandry. All animal studies were approved by the Michigan State University IACUC. Rats were housed in AAALAC-accredited facilities, in accordance with the *Guide for the Care and Use of Laboratory Animals*.[26] Rats (n = 24; 12 female, 12 male; age, 6 to 8 wk; donated from an inhouse colony of strain BDNF [SD-*Bdnf*^{em1Sage}] used in the study of Alzheimer disease) were housed under a 12:12-h light:dark cycle at a density of 2 rats per polysulfone microisolation cage (NexGen Rat 900, Allentown Caging, Allentown, NJ). Housing rooms were maintained at 68 to 72 °F (20 to 22.2 °C), with recorded relative humidity of 39% to 58% during the days of experimentation. Rats were housed on disposable bedding (aspen chips, Northeastern Products, Warrensburg, NY) and provided with enrichment tubing. Wire-lid food hoppers were filled with rodent chow (Teklad Global Diets Irradiated 22/5 Rodent Diet 8940, Envigo, St Louis Mo), and rats received reverse-osmosis–purified water via a rack-level watering system; chow and water were available without restriction. Quarterly sentinel samples are tested at a commercial laboratory (IDEXX BioAnalytics); specifically, blood, feces, and fur samples are submitted for serology, parasitology, and PCR testing as appropriate. According to this approach, the animals in the study were free of the following pathogens: parvovirus (Toolan H1 virus, Kilham rat virus, rat parvovirus, rat minute virus), coronavirus (rat coronavirus, sialodacryoadenitis virus), rat theilovirus, *Pneumocystis carinii*, Sendai virus, pneumonia virus of mice, reovirus type 3, *Mycoplasma pulmonis*, lymphocytic choriomeningitis virus, mouse adenovirus, Hantaan virus, *Encephalitozoon cuniculi*, and rotavirus (infectious diarrhea of infant rats virus).

Temperature transponder placement. Prior to the experimental surgery, rats were anesthetized briefly by using inhaled isoflurane for placement of subcutaneous temperature transponders (IPTT-300, BMDS, Seaford, DE) that transmit body temperature and a unique ID number to a scanning device system (DAS-6007, BMDS). Anesthesia was induced by using isoflurane (3% to 4% in O_2 at 0.4 to 0.6 L/min) in a 2-L plastic induction box. Rats were deemed fully anesthetized when righting reflex and withdrawal reflex, as demonstrated by no response to toe pinch, were lost. Isoflurane was reduced to 1.5% to 2% in 0.4 to 0.6 L/min O_2 for maintenance of anesthesia by using a nose cone for approximately 5 min (SomnoSuite, Kent Scientific, Torrington, CT). All microchip transponders were injected subcutaneously between the scapulae as directed by the manufacturer and successfully provided data during the course of the experiments. Rats recovered from anesthesia and were returned to social housing for a period of acclimation prior to surgery.

Prewarming treatments. After a 5- to 6-d recovery period from subcutaneous temperature transponder placement, rats were randomly divided into groups (n = 6 per group; 3 male and 3 female rats in each group) and assigned to 1 of 4 prewarming treatments prior to undergoing a cranial procedure to mimic a craniotomy. Prewarming treatments were implemented prior to cranial incision and included: no prewarming treatment (Control), warm-water blanket (WWB) under the cage to prewarm the cage (PW, blanket heated to 42 °C), WWB under the cage (heated to 42 °C) plus warmed intraperitoneal fluids (PWF, fluids heated to 36–38 °C), or warmed intraperitoneal fluids only (F).

Rats randomized to receive fluids were removed from warmed cages (PWF) or from unheated cages (F), underwent induction of anesthesia with isoflurane, were moved to the prep station, and were placed on a nosecone to maintain anesthesia. Once anesthetized, sterile 0.9% NaCl (2.0 mL IP, warmed to 36 to 38 °C) was then administered. For the prewarming process, awake rats in the PW and PWF groups were placed individually in a clean static unbedded cage; cages were placed on WWB once the water-pump light showed as 'ready,' indicating that 42 °C (the highest available setting) had been reached. Cages were kept on WWB for 30 min to provide whole-body thermal support to the awake rats prior to cranial incision. Control animals and those receiving only warmed fluids (group F) were kept in clean static unbedded cages that were placed on top of an unheated WWB for 30 min. Transponder temperatures were collected at 0, 15 and 30 min during the prewarming phase. In addition, surface temperatures inside control and prewarmed cages, without animals, were collected (model 905-T2, Surface Thermometer, Testo, West Chester, PA); temperatures were taken at the center of the cage floor and from the 4 cage corners and then averaged to accommodate slight heating variations that may occur across the WWB surface area.

Skin preparation and surgical procedure. All surgeries were performed by 2 persons (JJK, JMD) in a dedicated procedure room and no other activities were ongoing in the room during surgery periods. Environmental parameters of the procedure room were recorded at the start of each day when surgeries were performed (21 to 22 °C and 54% to 65% humidity across the experimental period). To handle rats, personnel wore disposable lab coats, surgical masks, disposable hair bonnets, and nonsterile nitrile exam gloves. Anesthesia was induced by using isoflurane (3% to 4% in O_2 at 0.4 to 0.6 L/min) in a 2-L transparent plastic induction box. Once the righting reflex was lost, rats were weighed on a gram scale and then placed in sternal recumbency on a cloth pad overlying a circulating WWB set to 38 °C. Isoflurane was administered at 1.5% to 3% via nose cone to maintain a surgical plane of anesthesia. Respiratory rate was monitored visually, and firm manual pressure was applied periodically to the metatarsals of the hindfeet to assess for the absence of the pedal-withdrawal reflex, which indicated the desired surgical anesthetic plane. During surgical prep, some animals were temporarily transitioned to dorsal recumbency and received intraperitoneal fluids if assigned to that intervention grouping.

Rats received a dose of meloxicam (2 mg/kg SC; Eloxiject, Covetrus, Dublin, OH) while anesthetized at the prep station, prior to hair clipping; a second dose of analgesic was given 24 h later. Sterile eye lubricant (Artificial Tears Solution, Covetrus) was applied to both eyes. Intraoperative temperature recordings were obtained by using a channel thermometer (BIO-TK9882, Bioseb In Vivo Research Instruments, Pinellas Park, FL) and rodent rectal probes (BIO-BRET2, Bioseb In Vivo Research Instruments). Once the rat was positioned in sternal recumbency at the prep station, a thermometer probe was inserted into the rectum. Temperature readings were automatically recorded every minute until completion of surgery.

A 3 × 3-cm area of hair centered between the ears and on the cranial midline was removed by using clippers and a number 30 blade (Wahl Clippers, Sterling, IL). The WAB agent used in our previous mouse study (61% ethanol plus 1% chlorhexidine

gluconate; Avagard Surgical and Healthcare Personnel Hand Antiseptic, 3M, Saint Paul, MN) was applied to rats.[13] This WAB antiseptic is the only FDA–NDA-approved brushless, waterless agent that provides emollients for skin conditioning. The WAB agent was dispensed directly from the original container at the time of surgical skin preparation; specifically, the spout on the front of the bottle is turned clockwise until it points downward, and manual pressure on the pump attachment port at the back of the bottle releases agent from the spout. A single pump volume of WAB from the container delivered directly to a sterile gauze square was applied to the clipped cranial area in a circular manner, starting from the center and expanding in concentric circles outward, to cover the clipped skin to the hairline border (Figure 1 A and B). Contact time for WAB antiseptic was approximately 1 min to allow for full drying of agent prior to a second application; this practice both ensured that the surgical site was fully dry for bacterial culture swabbing (described later) and prevented excessive wetting of the skin. The WAB stock bottle was kept at room temperature in the surgical suite.

After hair clipping, a baseline culture swab was collected from each rat by using a sterile culturette (ESwab Collection and Transport System, Becton Dickinson, Franklin Lakes, NJ) rubbed horizontally across the totality of shaved skin. Additional swabs were similarly collected after WAB agent was applied and again at postoperative skin closure (n = 3 cultures total per rat). Cultures were collected by rubbing the swab across the skin of the previously defined clipped skin area, with care taken to prevent contact of the swab with the bordering haired skin or any other surfaces before placement into the transport sheath. Culture swabs were submitted to IDEXX BioResearch (Columbia, MO) for aerobic culture and identification of bacterial growth by matrix-assisted laser desorption–ionization time-of-flight (MALDI-TOF) mass spectrometry.

Next, each rat was moved from the prep area to the surgery station and positioned into a stereotactic device, with stabilization of the head by using ear bars (Figure 1 C). Rats were placed on a heat source (E-Z Heat Surgical Bed Warming System, E-Z Systems, Palmer, PA) set to 38 °C, which was overlaid with a disposable cloth pad and autoclaved cloth drape to prevent direct contact of the heat source with the rat's skin. Appropriate depth of anesthesia was confirmed by absence of toe pinch reflex after repositioning. An approximately 2-cm–long cranial incision was made on the dorsal midline, through the fascia, to expose the underlying skull. The cranium was left exposed, as though in preparation for cranial injection for experimental treatments, for 10 min; skin was then closed by using 9-mm stainless steel wound clips (9-mm EZ Clip, Stoelting Company, Wood Dale, IL). After anesthesia was discontinued, the ear bars of the stereotactic apparatus were removed, and rats continued to receive O_2 by nose cone until the first purposeful movement was observed, at which time the rectal temperature probe was gently removed. Rats remained on the heating bed until return of righting reflex and then were transferred to a recovery cage placed partly onto a WWB set to 38 °C. The entire surgical procedure lasted approximately 40 min per rat. The surgical site was swabbed at the time of skin closure with a sterile culturette as described above. Rats were monitored continuously during recovery from the anesthetic event.

Postoperative period. Once fully recovered, rats were placed in clean, bedded cages and returned to the same caging conditions in the same room in which they were housed prior to the procedure. Rats were returned to their social housing, and in no case did cage mates disrupt the surgical sites of operated rats. Rats were assessed daily after surgery, with subcutaneous temperature readings and body weights collected. On the third postoperative day, rats were euthanized by 30% volume displacement per minute to achieve CO_2 narcosis (per expected adherence to AVMA guidance)[26] followed by induced bilateral pneumothorax as a confirmatory method.

Bacterial culture and identification. Bacterial colonies were cultured, enumerated, and identified by MALDI-TOF mass spectrometry, as previously described.[13,37] Representatives of each isolated colony morphology were selected for proteomic analysis, harvested, and transferred by using a sterile toothpick, overlaid with 1 µL of HCCA matrix (a saturated solution of α-cyano-4-hydroxycinnamic acid in 50% acetonitrile, and 2.5% trifluoroacetic acid; Bruker Daltronics, Billerica, MA), allowed to air dry at room temperature, and analyzed by MALDI–TOF by using a mass spectrometer (Microflex, Bruker Daltronics) and flexControl software (Bruker Daltronics). The time-of-flight of microbial proteins to the detector is a direct function of the mass:charge ratio (m/z) of each protein, forming the basis of a spectrum that functions as a molecular fingerprint of proteins in each bacterial isolate. Genus- and species-level identification of each isolate was based on automated analysis by MALDI BioTyper software (Bruker Daltronics), which compared the spectra for each isolate with an integrated reference database.

Statistical analysis. Descriptive statistics were performed regarding the recovery time of rats that had received prewarming on a WWB (PW), intraperitoneal delivery of warmed fluids (F), or prewarming and fluids (PWF). One-way ANOVA modeling was applied by using the following equation:

$$y_{ij} = \mu_i + \epsilon_{ij},$$

where i = 1,…,4; j = 1,…,6. In the model, y_{ij} is the recovery time of rat j on treatment i; μ_i : average recovery time of treatment i; ϵ_{ij} : normally distributed random errors. A further posthoc test was applied to determine whether any of the thermal treatments differed from the control group.

One way ANOVA was similarly applied to determine any relationship between recovery time and weight of the rats. To assess weight distribution across groups, the Kolmogorov–Smirnov test was applied to evaluate for differences in anesthetic recovery time when rats across all experimental groups were reorganized into groups based on body weight alone (100–199 g, 200–299 g, 300–399 g).

Results

Prewarming and intraoperative temperatures. Static cage environments were assessed over the 30 min of placement on an unheated or a heated WWB to estimate the floor temperatures to which rats would be exposed. Cage floor temperatures for the unheated WWB remained consistent with the ambient room temperatures (approximately 21.3 to 22.2 °C) over 30 min. Prewarmed cages (those placed on the WWB after the blanket reached 42 °C) were approximately 21.3 to 21.5 °C at time 0, increasing to 29.6 to 30.5 °C by 15 min, and heating further to 31.0 to 32.3 °C by 30 min.

The average body temperature of rats, obtained from subcutaneous transponder readings, across groups at the start of placement in empty cages was 37.2 °C. After 30 min of exposure to a warmed cage, PW and PWF rats had average body temperatures of 38 to 38.5 °C, whereas the control and F groups had average body temperatures of 37.8 to 38 °C. Over the intraoperative period of approximately 40 min, no significant differences in body temperatures occurred across treatment groups, based on rectal temperature readings (Figure

Figure 1. (A) Image of WAB agent on a cotton-tipped applicator. Due to the presence of emollients, the consistency of this agent is thicker than traditional chlorhexidine and povidone–iodine surgical scrub agents. (B) Surgical site of a rat with hair clipped, with first application of WAB antiseptic prior to spread in concentric circular fashion. (C) Position of rat within stereotactic device. Note: images in panels B and C show a representative rat and not one of the animals enrolled in the current study.

2). Temperature comparisons between rectal (core) thermometry and subcutaneous transponders were similar, with the average difference being a 0.43 °C higher reading from core measurements than from the subcutaneous transponder. Temperatures fell for all groups over time during surgery, with an average body temperature loss of 1.34 °C across all treatment groups. The lowest body temperature experienced was 34.4 °C in one rat from the control group, just prior to the first purposeful movement after discontinuation of isoflurane.

The 2 groups that received fluids (PWF and F) showed the greatest increase in standard deviations of the mean of body temperatures at the time of fluid administration. The body temperatures in the PWF group at the start of surgical prep were not statistically different from the other groups (mean group temperatures at start of surgical prep: PWF 37.0 °C, PW 36.5 °C, F 36.1 °C, control 35.8 °C). All rats that received warmed fluids in our study showed signs of transient increased respiratory effort. Increased respiratory effort was most pronounced while rats were temporarily positioned in dorsal recumbency to allow for intraperitoneal injection of fluids and resolved within a few minutes of return to sternal recumbency.

Time to recovery from anesthesia. Time to recovery from anesthesia did not differ with regard to warming treatments (Figure 3). Recovery times (mean ± 1 SD) of the 4 groups were: control, 8.5 ± 3.0 min; F, 8.2 ± 2.3 min; PW, 6.7 ± 14 min; and PWF, 8.7 ± 3.0 min. On the day of surgery, female rats ranged in body weight from 147–199 g, and males weighed 179–389 g. As

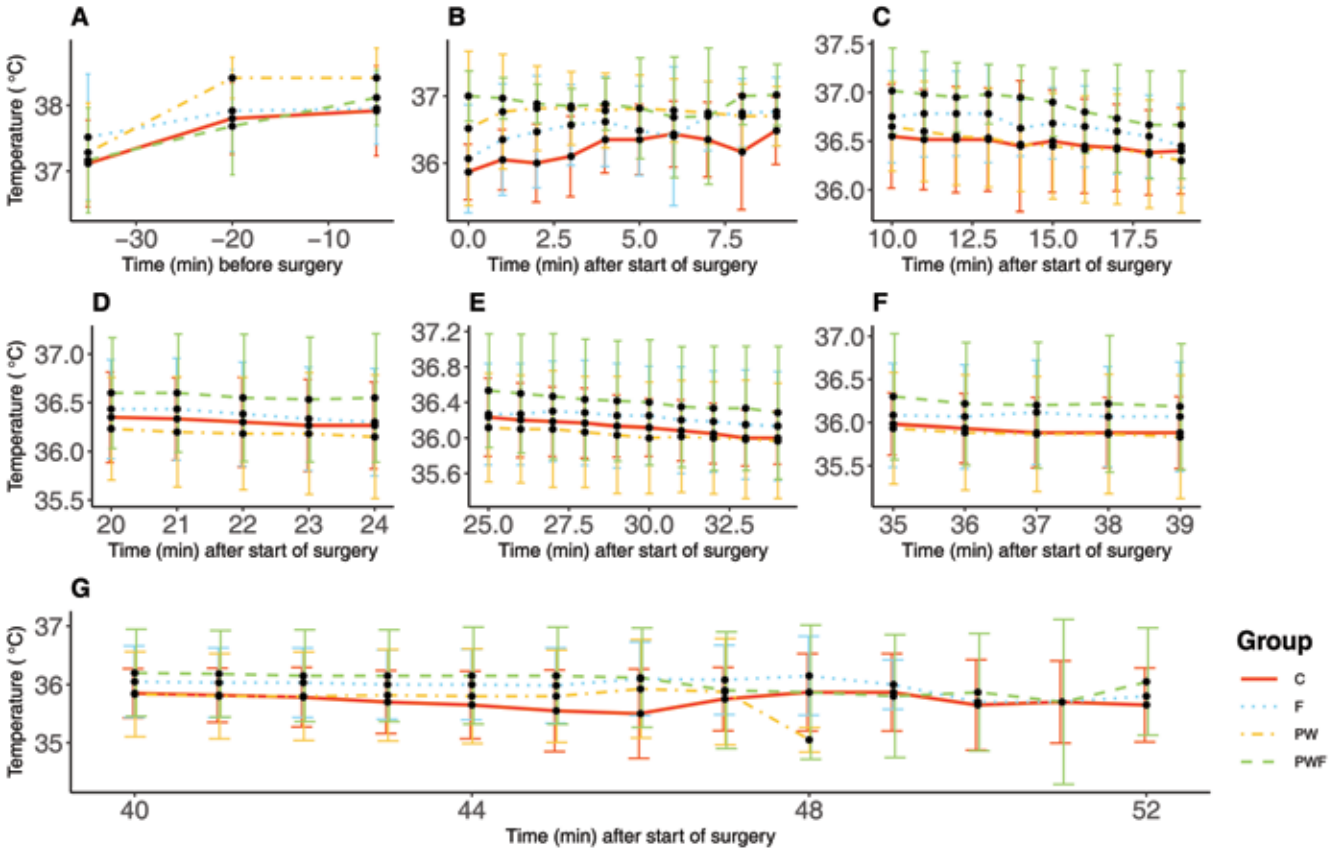

Figure 2. Average subcutaneous (A) (baseline phase only) and rectal (all other phases (B) start prep, (C) begin scrub, (D) begin surgery, (E) incision exposure, (F) incision closed, (G), isoflurane off) temperature at each minute according to prep group during phases of the surgical procedure until anesthetic recovery. The rectal probe was removed at the time of the first purposeful movement. C, control group; F, fluids-only group; PW, prewarming group; PWF, prewarming plus fluids group.

compared with prior work,[13] rats took longer to recover from anesthesia than did mice.

Recovery time differed significantly ($P < 0.05$) when all rats were grouped by body weight instead of experimental treatment, with rats in the heaviest weight group (300–399 g) recovering significantly faster than rats in the lightest weight group (100–199 g) (Figure 3). Rats in the heaviest weight group were all males whereas the lightest weight group was composed primarily of female rats, corresponding to the sex-associated growth differences in rats. The lightest weight group (100–199 g; mean 178 g; SD 15 g) contained 12 female and 2 male rats, the middle weight group (200–299 g; mean 255 g; SD 28 g) contained 7 males, and the heaviest weight group (300–399 g; mean 381 g; SD 6 g) contained 3 males. The recovery times (mean ± 1 SD) of animals within the 3 weight categories were: 100–199 g, 8.8 ± 2.1 min; 200–299 g, 7.7 ± 3.1 min; and 300–399 g, 5.7 ± 1.0 min.

Microbiologic assessment. Given its ability to both sustain body temperature and provide antisepsis in mice, we used the same WAB agent in all of the rats to assess antimicrobial activity on skin in rat. Aerobic culture and MALDI-TOF mass spectrometry identified 12 bacterial species and unspeciated bacteria from *Bacillus*, *Corynebaterium*, *Paenibacillus*, and *Psychrobacter* (Table 1). Numerical counts (that is, number of colony forming units; cfu/100 µL) were obtained from swabs (cumulative total cfu from all animals: after hair clipping - 56 cfu, after 2 applications of WAB agent - 1 cfu, after skin closure - 3 cfu), and for the various genera detected, the highest number of rats ($n = 7$) had positive cultures of *Paenibacillus*. Among all 24 rats enrolled in the study, 9 (approximately 38%) had no bacterial growth cultured from the cranial incision site at any point before or after WAB antiseptic application.

Postoperative assessments. Rats were maintained for 3 additional days after the cranial incision procedure. Subcutaneous temperature and body weight measurements were collected daily. All postoperative subcutaneous temperature measurements across all treatment groups were within the normal temperature range for rats, indicating that prewarming treatments and surgery did not have prolonged effects on body temperature. Although some rats (1 male in the control group; 2 males and 1 female in the PW group, and 2 males and 1 female in the PWF group) experienced mild postoperative weight loss, average body weight changes in all group trended toward weight gain over the 3 d after surgery, with prewarmed rats appearing to maintain weight (Figure 4). When body weights were compared between rats weighing 100–199 g and 200–299 g, 100–199 g and 300–399 g, and 200–299 g and 300–399 g, the P values obtained were 0.216, 0.738, and 0.107, respectively. Null hypotheses were not rejected; therefore when grouped by body weight, all had the same distribution at a significance level of 0.05, thus confirming that weight groups of rats were evenly distributed.

Discussion

To benefit our institutional research community, we sought to improve upon surgical support provided for rats undergoing procedures, particularly when placed in stereotactic equipment for cranial access. To validate WAB application as a replacement

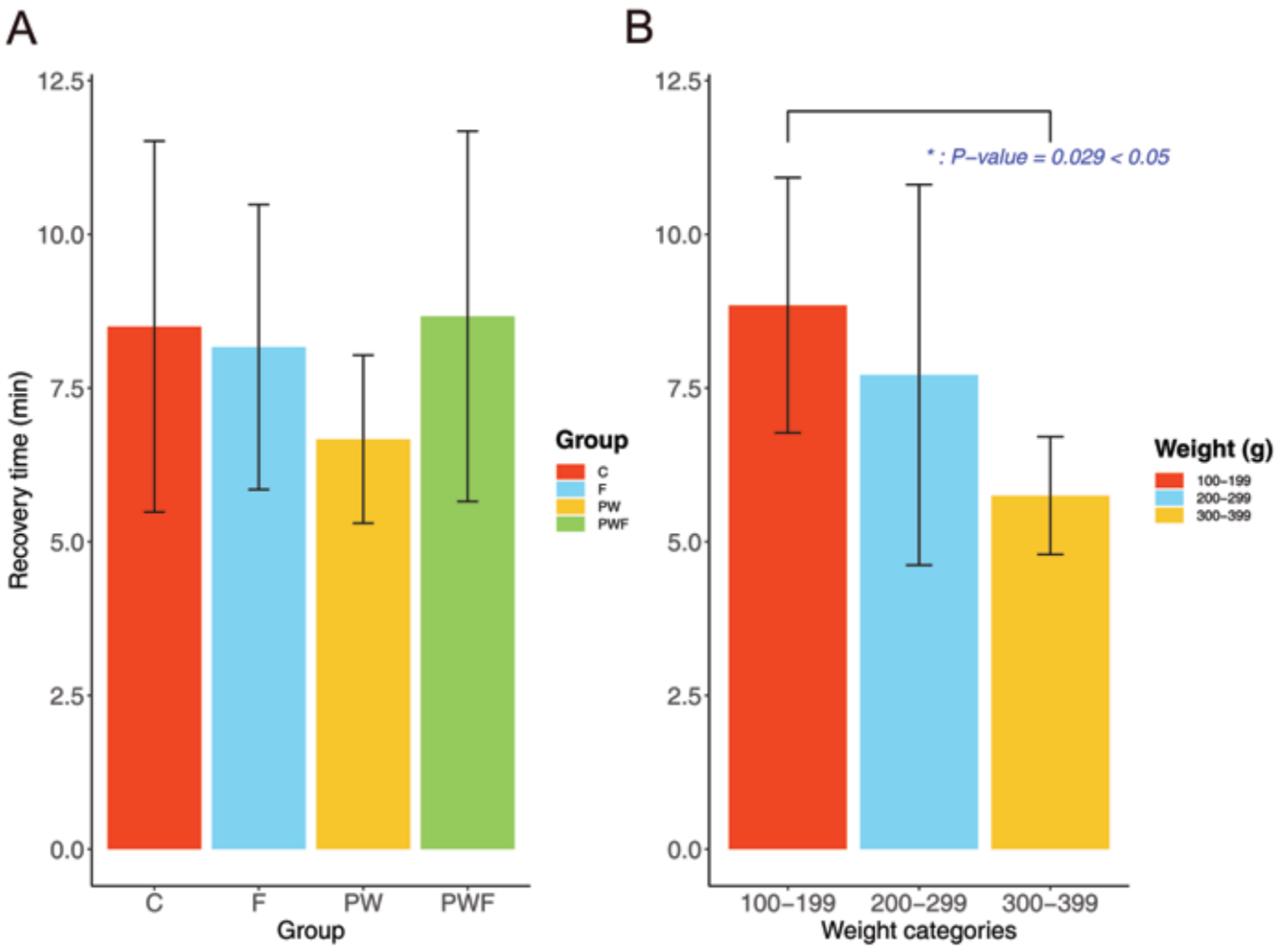

Figure 3. (A) Recovery time according to treatment group and rat weight. For all treatment groups of rats, recovery time (minutes) after discontinuation of isoflurane anesthesia was recorded. Rats that received the prewarming treatment (PW) of a heated cage for 30 min prior to anesthetic induction had the fastest average recovery time, whereas those that received PWF (prewarming and fluid) treatment and the control group had a longer time to recovery; however, average recovery times overall did not differ statistically ($\alpha = 0.05$). (B) In contrast, recovery time varied by rat body weight, with those in the lightest weight category (majority females) taking significantly longer to recover than those in the heaviest weight group (all males; $P \leq 0.05$). The height of the bar represents the mean value of the recovery time (minutes) of each group, and each side of the error bar is 1 SD away from the mean value.

to classic liquid skin prep protocols in rats, we made the decision to reduce the overall number of animals enrolled to only the minimum necessary to assess thermal interventions. We compared rat outcomes with WAB antiseptic with historical controls (that included povidone–iodine, ethanol, and chlorhexidine comparisons) from 2 prior mouse studies[13,44] and predicted a similar efficiency for rat skin disinfection. Cranial skin culture results demonstrated that this surgical site remains relatively uncontaminated with environmental bacterial species, despite rodent grooming behaviors that occur around the face and head. Specifically, in our cohort of rats, multiple animals (38% overall; 6 of 12 female rats and 3 of 12 males) had no bacterial agents detected at any perioperative time point (baseline, after skin preparation, and after skin closure). Those bacteria that were detected from rats in this study were expected skin microbiota, as well as potential contaminant species from human interaction with the animals and room environment.[9,18,19,31,45] Following the duplicate application of WAB, which was allowed to dry between applications with no rinse, bacterial counts were essentially reduced to 0% on the skin. Rats experienced an approximately 1 °C decrease in body temperature at the time of skin contact with WAB antiseptic; yet this change was not significant across treatment and control groups and was less than that reported to occur in mice (2 to 4 °C) when the same agent was applied to ventral skin.[44] As in mice, rats had no appreciable postoperative complications or significant temperature or body weight changes after surgery. In addition, an advantage of the WAB gel formulation is that when prepping the cranium, it does not escape from its placement at the surgical site and eliminates concerns of seepage into ears and eyes that might occur with liquid prep agents.

Recent studies in human and laboratory rodent patients have evaluated active prewarming measures to offset hypothermia and shivering responses. One study found that in pregnant women scheduled for caesarean section surgery, provision of forced-air blankets set to 41 °C for 15 min before surgery increased body temperature and reduced hypothermia. When the forced-air was coupled with warmed (41 °C) intravenous fluids intraoperatively, the women had significantly better maintenance of core temperatures throughout the epidural and surgical procedure.[28] In laboratory rats, forced delivery of warm air into a chamber environment promoted maintenance of core body temperatures and faster anesthetic recovery times; although warm air blowing directly onto faces of animals may cause a degree of stress.[40,42]

Table 1. Bacterial genera isolated from skin swabs after aerobic culture and MALDI-TOF identification (n = 24 rats).

	No. (%) of rats positive for this genus	Species cultured
Actinobacillus	2 (8.3)	*A. muris*
Bacillus	1 (4.2)	Unidentified species
Corynebacterium	1 (4.2)	Unidentified species
Enterococcus	3 (12.5)	*E. gallinarum*
Escherichia	1 (4.2)	*E. coli*
Lactobacillus	1 (4.2)	*L. murinus*
Micrococcus	2 (8.3)	*M. luteus*
Paenibacillus	7 (29.2)	Unidentified species
Psychrobacter	1 (4.2)	Unidentified species
Rothia	1 (4.2)	*R. nasimurium*
Staphylococcus	6 (25.0)	*S. aureus, S. cohnii*
Streptococcus	6 (25.0)	*S. danieliae*
Unidentified gram-negative rod	3 (12.5)	Unidentified species

Swabs were collected prior to and after WAB antiseptic application and after skin closure. Data reported represent all animals positive for a species of that genus on at least on culture. All rats had skin prepped similarly at the cranial incision site; 9 of 24 rats had no bacteria cultured at any time point.

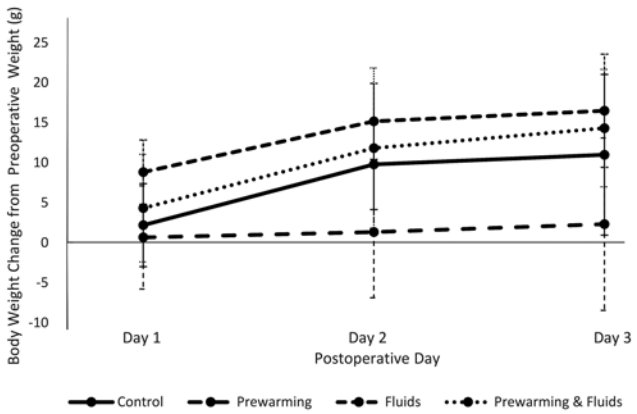

Figure 4. Average difference in body weight compared with preoperative body weight (BW) according to treatment group. All rats were weighed just prior to surgery and during postoperative checks at 1, 2, and 3 d after surgery. Each rat's weight on each postoperative day was compared with that same rat's preoperative weight to determine the net BW change. Individual BW differences were then used to calculate average differences in BW according to treatment group. Although some individual rats (1 male in the control group; 2 males and 1 female in the PW group; and 2 males and 1 female in the PWF group) experienced mild postoperative weight loss, average BW changes in all group trended toward weight gain over the 3 d after surgery. Errors bars represent 1 SD above and below the mean value.

Mice placed in a modified egg incubator prior to renal ischemia–reperfusion injury maintained a higher stable body temperature throughout the surgical procedure, resulting in increased kidney weights - indicating greater contralateral kidney response to the injury - and optimal data outcomes.[34]

Our data showed no significant decrease in time to postoperative recovery and righting reflex, regardless of whether rats were prewarmed, given warmed fluids, or remained in microisolation cages without additional heat. Over the prewarming phase of cages, the ambient chamber temperature increased by approximately 10 °C above that of control cages, yet rat body temperatures increased less than 1.0 °C during this time. Regardless of the thermal intervention, rats of greater body weight recovered from anesthesia significantly more quickly than lighter rats, likely due to the fact that animals with greater body mass have lower thermal conductance and increased insulation, enabling them to maintain warmer core temperatures overall.[21] To determine if other species (mice, humans) follow a similar recovery pattern, additional studies would need to be undertaken. Other means of active warming, such as by forced air and warming blankets, have been reported to help to preserve patient body temperatures during procedures,[30,39,42,51,52] as was demonstrated with our use of the heated platform throughout the surgical procedures for all rats.

Provision of fluids during surgery is common in medical practice, and often rodents receive a bolus dose of fluids to offset evaporative losses during surgery. Warming of fluids delivered to surgical patients benefits humans[5,8] but is less standard and may be relatively ineffective when delivered intravenously to veterinary patients.[15,27,44] Rats that received warmed intraperitoneal fluids in our study tended to show transient signs of increased respiratory effort; this effect was attributed to placement of the rats in dorsal recumbency to deliver the intraperitoneal fluid bolus just prior to placing animals in sternal positions on the stereotactic equipment. Overall, warmed intraperitoneal fluids did not show a statistically significant effect on body temperature maintenance. However, the adverse outcome of respiratory difficulty negated the apparent thermal benefits that otherwise can be achieved instead by external heat sources. Although not investigated herein, the delivery of warmed subcutaneous fluids might have less risk of side effects and could be useful in sustaining hydration for animals undergoing prolonged surgical procedures while under anesthesia. Direct warming of the skin preparation agents prior to application also might mitigate heat loss in veterinary patients, although this option was not evaluated as part of our project.[30,38]

In conclusion, adequate thermal support of rats undergoing cranial incisions can be accomplished by placing them on a heated surface during surgical procedures, without additional interventions. Even a modest improvement in return to consciousness after surgery can benefit animal health[32,43,44] and warrants further investigation of active warming methods provided to rodents that may undergo invasive and prolonged surgical procedures.

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