The Analgesic Effect of a Vapocoolant Stream Spray in Reducing Heat Nociception on the Glabrous Skin of Rat Pups

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BACKGROUND: Blood sampling is a common screening and diagnostic test in newborn infants in the neonatal intensive care unit, and heel lancing accounts for two-thirds of these tests. Heel lancing causes acute pain and distress, and most infants rarely receive analgesics because of fear of respiratory depression from opioids and lack of effectiveness of topical local anesthetics on the glabrous skin. To circumvent this latter problem, we investigated the analgesic efficacy and safety of a topical vapocoolant spray.

METHODS: Forty Sprague-Dawley rat pups aged 7 days old were randomly assigned to receive either vapocoolant or saline spray on the plantar hindpaws for 5 to 6 seconds. Forty-five seconds later, the paws were subjected to a modified hotplate test to quantify the nociceptive flexor withdrawal (NFW) thresholds before and after treatment with the sprays. Seven days later, the animals were euthanized and the hindpaws were examined histologically. A nested analysis of variance approach was used to account for the triplicate measurements per animal. A 2-tailed $P < 0.05$ was considered significant.

RESULTS: At baseline, there were no differences in the NFW thresholds between the 2 groups ($P = 0.22$). After treatment, these thresholds were significantly lower in both vapocoolant ($P < 0.001$) and saline ($P = 0.008$) groups relative to baseline values. The vapocoolant group demonstrated a significantly longer NFW latency time compared to the saline group ($P < 0.001$). All specimens in both groups were examined and showed normal skin histology.

CONCLUSIONS: Vapocoolant spray treatment of the glabrous skin is effective and safe after a single treatment. (Anesth Analg 2014;119:1367–72)

Intensive care of newborns and particularly premature infants has improved survival, therefore, considerably increasing the number of the diagnostic and therapeutic procedures performed.[1] Blood sampling is a common screening and diagnostic test in healthy and sick newborn infants in the neonatal intensive care unit (NICU). It is estimated that 1 to 21 heel lances or venipunctures a day are performed in preterm or ill neonates, and of these the heel lance accounts for 87%.[1,2] Heel lance is more commonly performed on newborn infants because the infant's heel has highly perfused and vascularized skin, and requires less time to perform. However, the peripheral veins are very small and aspiration of blood through a needle is difficult, requiring technical skills and resulting in a lower success rate.[3]

Therefore, venipuncture requires experience to perform and may not be practical in premature newborns particularly when frequent blood sampling is required.[4]

Heel lance is a seemingly minor procedure performed by puncturing the lateral aspect of the heel and squeezing the tissue to extract the capillary blood. The acute tissue injury can cause pain and distress. Although painful heel lancing is performed daily, most reports indicate that analgesia is rarely administered, or is underused in practice. Earlier studies reported that only approximately 5% of infants undergoing heel lance in the NICU received some form of analgesics.[5–7]

Several studies in human infants have demonstrated that exposure to noxious skin-breaking procedures including heel lance and surgery can alter short- and long-term somatosensory responses later in life.[8–11] The developing nervous system in infants is probably more vulnerable to long-term adverse neurodevelopmental changes than in older children.[12–15]

Topical local anesthetics are ideally suited for anesthetizing the skin in infants because most lack systemic side effects. While various effective and safe forms of topical anesthetics are available for infants, these anesthetics lack efficacy for the glabrous skin of the heel compared to nonglabrous skin.[16,17] Several morphological and physiological properties may account for the lack of effectiveness of local anesthetics. A control trial in neonates demonstrated a high microvascular blood flow of the heel skin compared to nonglabrous skin and speculated that the rapid vascular uptake is responsible for the high clearance of topical local anesthetic before it can reach subcutaneous nociceptors.[18]

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To circumvent the high vascularity of the heel glabrous skin in infants, we investigated analgesic efficacy and tissue toxicity of a rapidly acting and evaporative topical vapocoolant analgesic spray on the plantar hindpaw of rat pups (Pain Ease®, a medium stream spray composed of 1,1,13,-pentafluoropropane (95%) and 1,1,1,2-tetrafluoroesthane (5%) and manufactured by Gebauer Company, Cleveland, OH). We hypothesized that the vapocoolant spray will prolong the latency time (in milliseconds) of reflex flexion withdrawal of the hindpaw in response to heat pain.

METHODS

Test Materials

The study nozzle stream sprays were prepared by Pain Ease in identical 30-mL canisters labeled as "A" for Pain Ease or "B" for normal saline, and the metal canister of the study sprays was at room temperature between 21.2°C and 23.4°C.

Animals

With approval from the IRB and adherence to the Guide for the Care and Use of Laboratory Animals,[19] we used 40 awake Sprague-Dawley rat pups aged 7 days old, both male and female (Charles River Laboratories Wilmington, MA). They were housed in a room on a 12-hour light/dark cycle with free access to water and food. Rat pups were kept in cages with their littermates and dams.

Behavioral Assessment of Nociception

A single investigator, who was unaware of group assignment, applied the study spray drug "A" to the right paws and "B" to the left paws. The treatments were sprayed continuously, for 5 to 6 seconds, at a distance of approximately 5 inches from the plantar hindpaws and allowed to take effect over 45 seconds before subjecting each paw to the hotplate test.

Heat pain sensitivity to spray drugs was measured by changes in nociceptive flexor withdrawal (NFW) latency time (milliseconds) in contact with a hotplate using a modified hotplate test that has been used in our and others' previous infant rat pup studies.[20,21] Prolonged or lack of withdrawal response compared to saline spray was considered effective analgesia.

The rat pup was positioned so that its hind paw was placed on a 52°C (accuracy is ±0.1°C) hotplate (model 39D hotplate analgesia meter; IITC Inc., Woodland Hills, CA). The latency with which the paw was withdrawn was recorded.

Thermal withdrawal latency was determined as the duration in milliseconds between contact and until the rats lifted their paws away from the hotplate. If there was no withdrawal response after 12 seconds, the experimenter removed the tested paw to avoid tissue injury.[20] This test was repeated 3 times at 10-second intervals at baseline and 3 more times after treatment with study sprays. After completion of the study, rat pups were returned to their dams for breastfeeding for 7 more days.

Euthanasia

On the 7th day after termination of the experiments, rats were euthanized in an induction chamber with medical grade inhaled compressed 100% carbon dioxide gas. After the rat pups were unconscious and respiration ceased, both hindpaws were collected and specimens were preserved in formaldehyde for histopathological analysis.

Histopathology

Rat pup hindpaws were fixed in 4% formaldehyde and embedded in paraffin, and 3 to 5 micron sections were cut for hematoxylin and eosin stain.

The tissue was examined, and a representative cross-section of each of the 40 hindpaws was submitted for routine processing and paraffin embedding. Five-micron sections were cut from each of the 40 samples and stained with hematoxylin and eosin stain. A board-certified dermatopathologist who was unaware of group assignment performed histological analyses of the hematoxylin- and eosin-stained slides. Each section showed a representative cross-section with clear visible epidermis, appendageal structures, dermis, subcutis, nerve bundles, muscle, fibrous connective tissue, cartilage, and bone with bone marrow elements. All examined specimens showed normal histology of glabrous skin after vapocoolant application at 4-, 10-, 20-, and 40-fold magnifications.

Statistical Analysis

Rat pups were randomized (1:1) to either active spray (n = 20) or saline spray group (n = 20). Power analysis was conducted using an independent t-test with a 2-sided significance level of 0.05. A conservative expected baseline of 1500 ms was chosen as a result of previous studies.[20,21] This was an exploratory investigation using vapocoolant spray in rat pups to assess pain using NFW thresholds where no preceding studies have been performed. Since a previous study of topical amethocaine gel to reduce pain from heel prick blood sampling used effect sizes of 1.60 (4 ± 2.5) and 1.33 (2 ± 1.5) to detect a meaningful difference in pain from the placebo and treated groups with a standard deviation between 63% and 75% of the measurement used, a conservative effect size and standard deviation of 1 ± 1 was used for evaluation (500 ± 500 ms).[22] Therefore, power analysis indicated that a sample size of 20 rats per group provided 80% power to detect a mean difference of 500 ms between active spray and saline spray after treatment assuming a standard deviation of 500 ms (effect size = 1). Power calculations were determined using nQuery Advisor version 7.0 (Statistical Solutions, Saugus, MA).[23,24]

Differences in heat pain latencies between baseline and treatment were assessed in rats that received the vapocoolant and the saline spray groups as well as differences between the 2 groups with repeated-measures mixed effects analysis of variance (ANOVA). Hotplate reaction time to pain was considered the outcome variable and treatment and time as the 2 factors in the model. A nested ANOVA approach was used to account for triplicate measurements per animal. A 2-tailed $P < 0.05$ was considered significant. Statistical analysis was performed using SPSS version 21.0 (IBM, Armonk, NY).

RESULTS

Forty rat pups aged postnatal day 7 were used for this experiment. At baseline, there were no differences in the NFW thresholds between the 2 groups (P = 0.22) (Table 1, Fig. 1). After treatment, these thresholds were significantly

lower in both vapocoolant and saline groups relative to baseline values (Fig. 2). However, the vapocoolant group displayed a longer withdrawal threshold (700 ms difference, $P < 0.001$, between baseline and treatment) than the saline group (200 ms difference, $P = 0.008$, between baseline and treatment).

Two-way mixed effects ANOVA with the rat pup's reaction times to pain as the outcome variable and time and treatment as factors confirmed that both time (before or after treatment) and treatment (vapocoolant spray or saline group) were significantly predictive of NFW threshold (both $P < 0.0001$).

There were no pathologic changes identified in any of the 40 analyzed hematoxylin- and eosin-stained slides. The epidermis, dermis with appendageal structures, and nerve bundles were all carefully examined, and there were no differences noted between the vapocoolant and saline groups (Fig. 3, A–D).

Table 1. Characteristics of Hotplate Reaction Time (Milliseconds) in Rat Pups at Baseline and After Treatment[a]

Group	Baseline	After treatment
Vapocoolant	1400 ± 300	2100 ± 600
Saline	1300 ± 300	1500 ± 400
Vapocoolant and saline	1400 ± 300	1800 ± 600

[a]Values are presented in means and standard deviations.

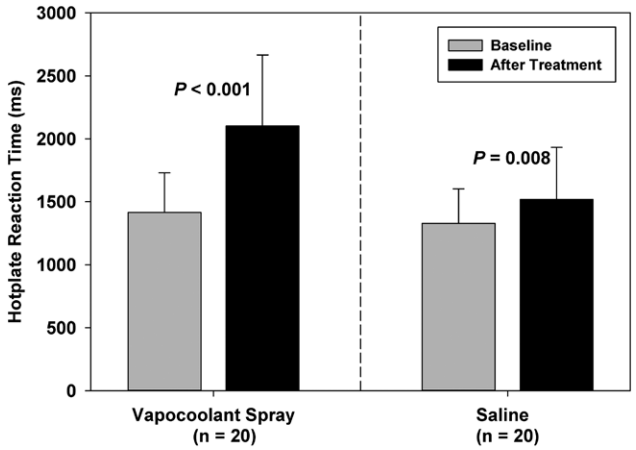

Figure 1. Comparison of nociceptive flexion withdrawal thresholds presented as mean reaction time in seconds at baseline and after treatment in vapocoolant (group A) and saline (group B) groups. There was no significant difference between the 2 groups at baseline, but there was a significant difference after treatment with vapocoolant compared to saline spray.

DISCUSSION

The main finding of this study is that the vapocoolant spray was effective for increasing heat pain thresholds compared to saline spray (Fig. 1). Heat nociceptive thresholds increased by a mean 700 ms relative to baseline values reflecting a reduction in pain behavior sensitivity after application of the vapocoolant spray, whereas the increase in heat nociceptive thresholds was a mean of only 200 ms with the saline spray (Fig. 1). Compared to saline spray, vapocoolant spray produced a 29% increase in the mean heat pain threshold. Only 1 pup rat (1/20) in the vapocoolant group had no change in threshold.

We demonstrated the effectiveness of vapocoolant spray for cooling the plantar glabrous skin to anesthetize subcutaneous nociceptors for a brief 5- to 6-second contact time. We also demonstrated that a single application of the vapocoolant spray did not cause cutaneous neural and histopathological changes.

Heel lancing produces acute cutaneous tissue injury, inflammation, and release of chemical mediators which activate and sensitize the peripheral heat and mechanical nociceptors.[25,26] In a longitudinal study of preterm infants born 27 to 32 weeks postconception age exposed to heel lancing, the repeated NFW threshold testing demonstrated a 50% reduction for the affected heel compared to the noninjured heel, indicating increased pain sensitivity on the side of heel lancing. When a topical cream (lidocaine and prilocaine eutectic mixture; EMLA) was applied to the damaged skin area, the hypersensitivity (measured by the NFW threshold) was reversed. However, placebo cream treatment had no effect.[27]

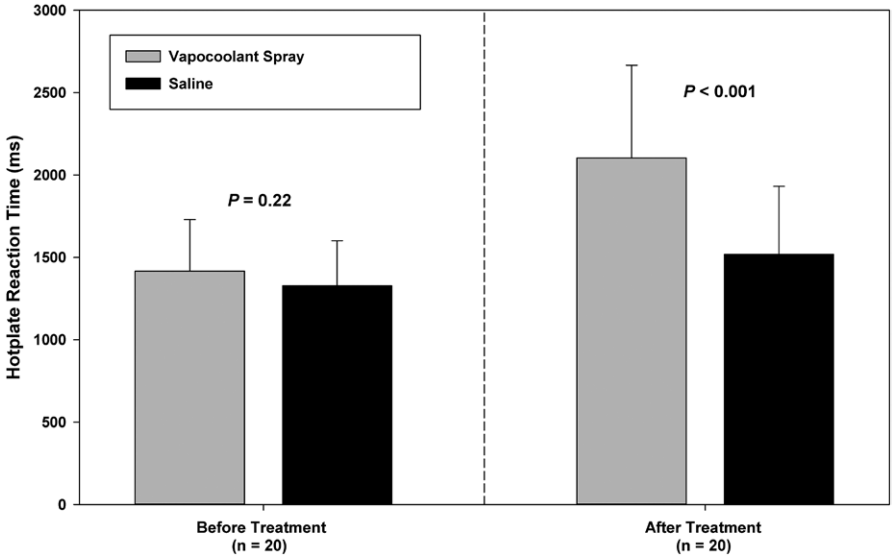

Figure 2. Within-group comparisons of changes in nociceptive flexion withdrawal thresholds presented as mean reaction time in seconds: vapocoolant (group A) and saline (group B) groups. Mixed ANOVA for heat pain latencies showed significant prolongation of flexion withdrawal thresholds in both groups.

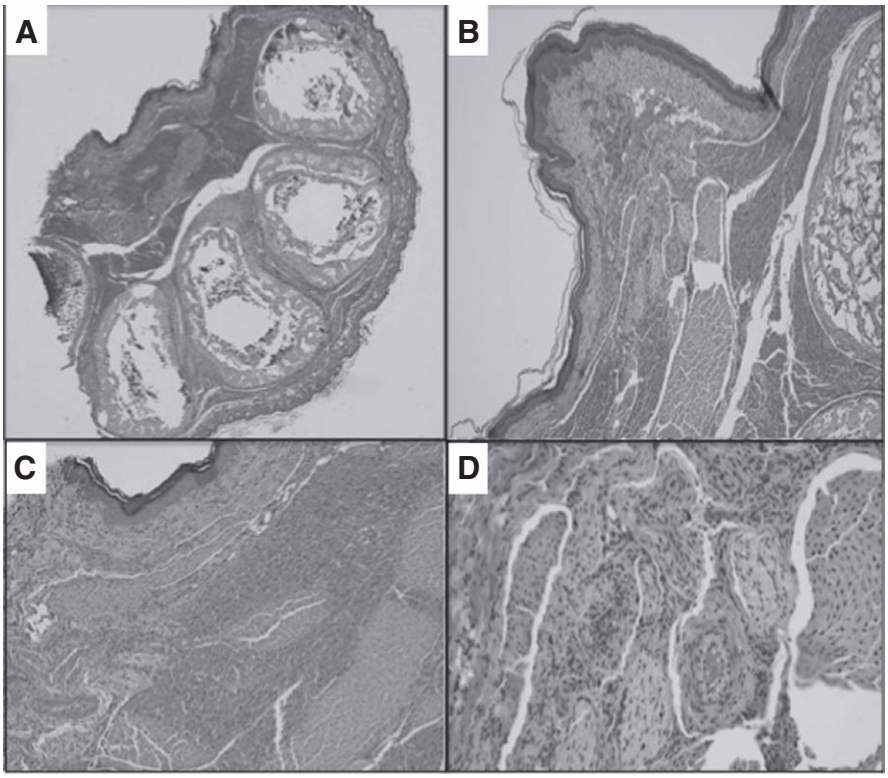

Figure 3. Rat Pup Paw. A, Low-power representative view of the rat paw showing: Epidermis, dermis with appendageal structures, blood vessels and nerve bundles, subcutis with bone and cartilage. B, Epidermis and dermis with nerve bundles and no pathologic changes and no inflammatory infiltrate. C, 20x power view shows the epidermis, papillary dermis and reticular dermis with prominent nerve bundles and no pathologic changes. D, High power images of nerve bundles and vascular structures within normal limits.

We elected to study rat pups on the 7th postnatal day because they are comparable to term human newborns.[28] Cutaneous NFW thresholds have been estimated in rat pups and human preterm and term neonates. These thresholds are low in both species and developmentally comparable with increase in age.[29] This reflex threshold is lowest and exaggerated in preterm infants (<32 weeks postconception age) and newborn rat pups (<2 postnatal weeks) probably due to immature descending inhibitory control.[29]

There are some similarities between rat pups and human newborns in the maturity of pain processing circuitry and parallel somatosensory developmental trajectories after birth. In general, the maturation of the nociceptive pathways in premature newborns of 24 weeks postconception age parallels that of rat pups at birth.[30] The neonatal spinal cord excitability, as measured by the flexion reflex withdrawal threshold, is present in both rat pups and human preterm and term neonates. This response diminishes (i.e., the threshold increases) with maturation of the somatosensory system beyond the newborn period. The flexion reflex withdrawal threshold (response time) to a painful stimulus also decreases similar to the responses to tenderness or hyperalgesia after tissue injury in adults.[31–33]

Pain Ease is marketed as a "non-drug" nonflammable topical anesthetic spray and is approved by the Food and Drug Administration for use on intact skin and mucous membranes and on minor open wounds. Its efficacy on nonglabrous skin has been demonstrated in a controlled randomized blinded trial in older children.[34] Spray application is recommended over 4 to 10 seconds or until the skin blanches at a distance of 3 to 7 inches to avoid frostbite. The duration of the vapocoolant analgesia is short-lived, approximately 60 seconds, because of rapid rewarming of the skin by cutaneous blood flow. A volunteer study involving application of vapocoolant stream spray to the forearm skin surface for 4 or 10 seconds duration and at 3 or 5 inches distance showed skin surface cooling below freezing temperature without tissue injury. Complete rewarming of the skin surface, subcutaneous and 1-cm intramuscular tissue, occurred within 4 to 5 minutes.[35]

The limitations of this study were that we did not first investigate the optimal efficacy at various stream spray durations of 4 to 10 seconds and distances of 3 to 5 inches. Second, we did not investigate the efficacy and safety of repetitive painful heel sticks per day to mimic a common practice in the NICU.

CONCLUSIONS

The findings from this preliminary study show that transient plantar skin cooling of rat pups with a single application of vapocoolant stream spray is effective and safe. While there are several anatomical and neurobiological parallels between rat pups and human infants, further experiments are needed to ascertain the safety and efficacy of the vapocoolant in response to a range of duration and distance of recommended applications after repeated daily applications to simulate the exposure to heel lances in NICU practice.

DISCLOSURES

Name: Navil F. Sethna, MD.
Contribution: This author helped design the study, conduct the study, and write the manuscript.
Attestation: Navil F. Sethna has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.

Conflicts of Interest: Navil F. Sethna received research funding from Gebauer Company, Cleveland, OH. Gebauer Company provided research funding and the Pain Ease® spray and placebo (saline) spray used in the study to Navil F. Sethna. This study was investigators initiated and independently conceptualized, designed, conducted, analyzed, interpreted the results, and prepared the manuscript.
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Attestation: Barak Yahalom has seen the original study data, reviewed the analysis of the data, approved the final manuscript, and is the author responsible for archiving the study files.
Conflicts of Interest: This author declares no conflicts of interest.
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Contribution: This author helped design the study, analyze the data, and write the manuscript.
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