The effects of aging on hydromorphone-induced thermal antinociception in healthy female cats

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Abstract

Introduction: This study aimed to evaluate the effects of aging on hydromorphone-induced thermal antinociception in cats.

Methods: In a prospective, randomized, blinded, controlled design, 10 healthy female cats received each of the following treatments intramuscularly: hydromorphone (0.1 mg/kg) and 0.9% saline (0.05 mL/kg) with a 1-week washout between treatments at 6, 9, and 12 months of age. Skin temperature and thermal thresholds (TTs) were recorded before and up to 12 hours after injection. Data were analyzed using a repeated-measures linear mixed model (α = 0.05).

Results: After saline treatment, TT was not significantly different from baseline at any time point for any age group. After hydromorphone treatment, TT was significantly higher than baseline at 6 months for up to 1 hour, and at 9 and 12 months for up to 4 hours. Peak TT at 6, 9, and 12 months were 50.4 ± 2.7, 50.9 ± 2.0, and 53.6 ± 2.0°C at 0.5, 1, and 1 hours, respectively. Mean TT was significantly higher after hydromorphone treatment when compared with saline treatment at 9 and 12 months for up to 4 hours but not at 6 months. Magnitude of antinociception was consistently larger at 12 months when compared with 6 months of age. Hydromorphone provided a shorter duration and smaller magnitude of antinociception at 6 months when compared with 9 and 12 months.

Conclusion: Pediatric cats may require more frequent dosing of hydromorphone than adults.

Keywords: Aging, Analgesia, Opioid, Pediatric, Thermal antinociception, Feline

1. Introduction

Opioids produce potent analgesic effects at the supraspinal (thalamus, hypothalamus, insular cortex, amygdala, cingulate gyrus, locus coeruleus, and periaqueductal gray) and spinal (laminae I and II of the dorsal horn) levels2,28 via the activation of G-protein–coupled receptors.15,18,43,44 These drugs are widely used in pain management in both humans and companion animals because of their great analgesic efficacy. In addition, opioid-induced adverse effects can be easily prevented or titrated in the clinical setting.16,23,34

Aging produces changes in the pharmacokinetics (ie, absorption, distribution, metabolism, and excretion) and pharmacodynamics (ie, receptor density, sensitivity, and maturation) of opioid analgesics.3,7,27,40,53 This could result in acute adverse effects with overdosing or lack of analgesia depending on doses and drug administered, and the patient’s age. Clinically, it is of utmost importance to know if opioid dose intervals should be tailored to aging and if, for example, pediatric individuals require more frequent opioid administration due to pharmacokinetic/pharmacodynamic differences than adult individuals. Identifying age-induced changes in the antinociceptive effects of opioids could lead to better opioid dosage regimens. However, there is currently a lack of studies on the subject.17,31,32,39,40 For example, adult rats (18–24 months of age) have demonstrated increased antinociceptive effects of µ- and κ-opioid receptor agonists when compared with young rats (3–6 months of age).22,40,48 Contrarily, reduced antinociceptive effects of opioids were observed in other studies as rats mature to adulthood.8,9,24,25,31,32 Due to the inconsistencies reported, additional research is required to determine the antinociceptive effects of opioids in pediatric and adult subjects. Ideally, the same group of subjects should be used to allow better data comparisons.
The primary objective of this study was to evaluate the antinociceptive effects of a single dose of \( \mu \)-opioid receptor agonist, hydromorphone, in female cats during aging as kittens, junior, and prime adults. According to the feline life staging guidelines,\(^{21} \) this would be equivalent to studying these effects on human pediatrics, adolescents, and adults. The primary hypothesis was that hydromorphone would induce greater, long-lasting antinociception as subjects aged.

2. Materials and methods

This study was approved by the Institutional Animal Care and Use Committee (IACUC) of the Texas A&M University College of Veterinary Medicine and Biomedical Sciences (IACUC 2016-0215). The manuscript is reported according to “Animal Research: Reporting of In Vivo Experiments” (ARRIVE) guidelines.\(^{26} \)

2.1. Animals

Ten healthy, research-bred (Liberty Research Inc, Waverly, NY), domestic, short-hair, female cats were included in the study. Health status was determined based on monthly physical examinations and annual complete blood count and serum biochemical analysis. Cats were dewormed and vaccinated for feline immunodeficiency virus and feline leukemia virus annually. All standards for housing and care were in accordance with the Texas A&M University College of Veterinary Medicine and Biomedical Sciences IACUC. Cats remained in a research facility under controlled light (12/12-hour light/dark cycle), temperature (21–22°C), and humidity (55%–60%) conditions and housed in 2 rooms of 2.4 m L × 2.4 m W × 3.0 m H. Environmental enrichment was achieved with the use of toys, scratch posts, condos and bedding. One wall consisted of large windows that provided natural light and the ability to visualize their surroundings. Food and water were available ad libitum but removed immediately before and up to 4 hours following treatment administration. During acclimatization and testing, cats were housed individually in adjacent cages (69.85 cm L × 69.85 cm W × 83.82 cm H).

2.2. Acclimatization

Kittens arrived at the research facility at 3 months of age. They were visited by the investigators daily during the study and acclimatization included gentle handling, playing, environmental enrichment, and positive reinforcement with food. Acclimatization also included settlement of cats into housing and cages, hair clipping, placement of the thermal threshold (TT) device, and testing 28 days before the study had begun at 6 months of age. Behavioral reactions were recorded for each cat during this period to familiarize the investigator with the cats’ response to thermal nociceptive testing. The same procedure was repeated during 14 days before the second (9 months of age) and third (12 months of age) testing periods.

2.3. Thermal nociceptive threshold testing

A TT device (Topcat Metrology, Ltd, Cambridgeshire, England) was used to evaluate thermal nociception.\(^{12} \) The device was calibrated and maintained according to the manufacturer’s recommendations. Detailed description on the use of this device has been reported elsewhere.\(^{38} \) In brief, a probe of approximately 1 × 0.3 cm was placed against the skin on the lateral aspect of the shaved thorax. This probe contained a heating component and a temperature sensor, and it was held in place using an elasticated vest containing the wireless receiver, power supply, and the probe itself (Fig. 1). Thirty minutes of direct skin contact with the probe were allowed prior to testing. Immediately following skin temperature (ST) recording, the evaluator triggered a ramped heat stimulus of 0.6°C/s via a handheld infrared remote device. The noxious stimulus was stopped when a nocifensive behavioral response was observed (ie, flinching, vocalization, turning toward the heating probe, jumping, and/or rolling) or when the cutoff temperature of 55°C was reached to prevent burns.\(^{13} \) The temperature recorded was considered the TT.

Skin temperature and TT testing were evaluated before (baseline) and at 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 10, and 12 hours after treatment administration. Baseline values were determined using the mean of 3 recordings performed at 15-minute intervals before treatment administration. Skin temperature and TT testing were performed at 6, 9, and 12 months of age for all cats. A single observer (B.T.S.) was blinded to the treatment allocation and performed TT testing. The device was removed for approximately 1 hour during the 2-hour time intervals to allow the cats to rest. Adverse events and additional observations were recorded during the testing period.

2.4. Treatments

In a prospective, randomized, blinded, controlled design, 10 cats were administered either hydromorphone hydrochloride (2 mg/mL; West-Ward, Eatontown, NJ) at 0.1 mg/kg or 0.9% saline solution (Covidien, Saint-Laurent, Canada) at 0.05 mL/kg in the epaxial muscles at 6 (kittens), 9 (junior), and 12 (prime adults) months of age. Randomization was performed using online software (www.randomization.org). The dose of saline was calculated so that both treatments were of equal final volume. Cats were placed on an examination table, and all treatments were administered by 2 individuals (B.T.S. or E.M.S.) who were blinded to treatment allocation. A 7-day washout period was allowed between treatments within each age period.

3. Statistical analysis

A power analysis revealed that a minimum of 8 cats would be needed to detect a significant difference between treatments with a TT difference of >2.5°C (\( \alpha = 0.05; \beta = 0.05 \)).\(^{38} \) Ten cats were included in the study due to the large individual variability in thermal nociception reported in previous studies.\(^{14,38} \) Data were evaluated for normal distribution by use of the Shapiro–Wilk test. The effect of time and treatment on TT for each age group and their interactions were analyzed using a repeated-measures linear mixed model. Treatment and time were considered fixed effects, and the cat was considered as a random effect. Body weight and ST were added as cofactors to the model. A series of contrasts were performed to examine differences between pairs of means adjusting the alpha level for each comparison with the Benjamini–Hochberg sequential adjustment procedure. For the time points in which TT was significantly increased from baseline values, the magnitude of antinociception was calculated using delta TT (ie, TT at each time point minus TT at baseline). The effects of time and age on delta TT were also evaluated using a linear mixed model with ST as a cofactor with similar sequential adjustment. The frequency of adverse events was compared between the 2 treatments using the Mantel–Haenszel \( \chi^2 \) test for repeated measures. Significance was set at \( \alpha = 0.05 \). Analyses were performed using SAS 9.4 (SAS 9.4 Software; SAS Institute, Inc).
4. Results

All cats completed the study at the age periods of 6, 9, and 12 months. Descriptive statistics of body weight and ST are available in Table 1.

4.1. Effect of treatment and time for each age group

Body weight was not associated with TT in any age group ($P > 0.10$, $P > 0.26$, and $P > 0.90$ for ages 6, 9, and 12 months, respectively). Skin temperature was not associated with TT in any age group ($P = 0.98$, $P = 0.10$, and $P = 0.58$ for ages 6, 9, and 12, respectively).

After treatment with hydromorphone, TT significantly increased at 0.25 hour ($P < 0.0001$), 0.5 hour ($P < 0.0001$), 0.75 hour ($P < 0.0001$), and 1 hour ($P = 0.0007$) than baseline at 6 months of age. Thermal threshold significantly increased at 0.25 hour ($P < 0.0001$), 0.5 hour ($P = 0.0016$), 0.75 hour ($P < 0.0001$), 1 hour ($P = 0.0007$), 2 hours ($P = 0.0009$), 3 hours ($P = 0.0003$), and 4 hours ($P = 0.013$) than baseline at 9 months of age. Thermal threshold significantly increased at 0.25, 0.5, 0.75, 1, 2, 3, and 4 hours ($P < 0.0001$ for all) than baseline at 12 months of age. After treatment with saline, TT was not significantly different than baseline at any time point or age group ($P > 0.06$, $P > 0.07$, and $P > 0.32$ at 6, 9, and 12 months, respectively).

When saline and hydromorphone were compared, there were no significant differences in the TT at 6 months of age ($P > 0.01$; not significant after adjustment). However, TT was significantly higher after the administration of hydromorphone when compared with saline at 0.25 hour ($P < 0.0001$), 0.75 hour ($P < 0.0001$), 1 hour ($P < 0.0001$), 2 hours ($P < 0.0001$), 3 hours ($P = 0.012$), and 4 hours ($P = 0.009$) at 9 months of age and at 0.25, 0.5, 0.75, 1, 2, 3, and 4 hours ($P < 0.0001$) at 12 months of age (Fig. 2). The magnitude of effect after hydromorphone administration calculated as delta TT is shown in Table 2. Skin temperature was not associated with delta TT ($P = 0.08$). Delta TT significantly increased at 1 hour ($P = 0.0045$) at 12 when compared with 6 months of age and at 2 hour ($P = 0.002$) at 12 when compared with 6 months of age.

4.2. Frequency of adverse events

The frequency of adverse events was higher in cats after hydromorphone treatment when compared with saline treatment (Table 3). There was no effect of age in the frequency of any adverse event after hydromorphone administration (vomiting, $P = 0.55$; licking or hypersalivation, $P = 0.09$; euphoria or dysphoria, $P = 0.74$). Adverse events after saline treatment were rare; thus, a statistical analysis on the effect of age was not performed.

5. Discussion

This study evaluated TT of cats as they became older after the intramuscular administration of hydromorphone or saline. Overall, there were significant differences in the duration and magnitude of antinociception between 6 and 9, and 12 months of age. After the administration of hydromorphone, the onset of antinociception was similar for all ages (ie, 15 minutes); however, the duration of antinociception was shorter at 6 months than at 9 and 12 months of age (ie, 1 hour vs up to 4 hours, respectively). The magnitude of antinociception was consistently larger at 12 months of age when compared with 6 months. Onset, duration, and magnitude of antinociception after hydromorphone administration were similar at 9 and 12 months of age.

5.1. Magnitude of hydromorphone-induced antinociception

The effects of age on opioid-induced magnitude of antinociception have been previously evaluated in rats and mice. It is difficult to draw relevant conclusions based on

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**Table 1**

Descriptive statistics for body weight and skin temperature of female cats undergoing thermal nociceptive testing at 6, 9, and 12 months of age.

| Age      | 6 months (n = 10) | 9 months (n = 10) | 12 months (n = 10) |
|----------|------------------|------------------|--------------------|
| Body weight (kg) | 2.8 (2.8–2.9) | 3.4 (3.3–3.5) | 3.4 (3.3–3.5) |
| Skin temperature (˚C) | 37.3 (37.2–37.4) | 37.1 (37.0–37.2) | 37.3 (37.2–37.3) |

Data are presented as mean (95% confidence interval).
previous studies mostly because of differences in study methodology. However, opioid-induced antinociception increases from young to adult rats and decreases from adult to geriatric rats. For example, the intraperitoneal administration of morphine was decreased in geriatric rats (27–31 months of age) when compared with adult rats (12–16 months of age) during thermal stimulation with a hot plate.32 However, this study did not evaluate the effects of opioids on young rats (<6 months of age). Similar results were also reported in geriatric rats (25–26 months of age) after an intrathecal injection of μ-receptor opioid agonist (D-Ala²,N-methyl-Phe⁴,Gly⁵-ol) encephalin (DAMPGO).8 DAMPGO-induced antinociception declined with age, and geriatric rats required approximately 30 times higher doses to achieve the effective dose (ED)₅₀ when compared with young rats (5–6 months of age). Contrarily, tail flick latencies were higher in adult rats than in young rats using the highest dose of DAMPGO. A previous study reported increased antinociceptive effects in adult rats (18 months of age) after the administration of morphine when compared with young rats (6 months of age).22 The latter is consistent with the present study in which junior (9 months of age) and adult (12 months of age) cats had significantly higher magnitude of hydromorphone-induced thermal antinociception when compared with kittens (6 months of age).

5.2. Duration of hydromorphone-induced antinociception

Few studies have reported the effects of aging on the duration of antinociception following the administration of opioids using the same subjects overtime.20,51 In the present study, the duration of hydromorphone-induced antinociception was significantly longer at 9 and 12 months when compared with 6 months of age. A previous study showed that the duration of morphine-induced antinociception was significantly shorter in geriatric mice (24–27 months) when compared with mature adult mice (3–6 months) (1.5 hours vs 3 hours, respectively).20 However, the authors are not aware of other studies evaluating the duration of opioid thermal antinociception in pediatric and young vs adult and geriatric subjects. In this case, extrapolations are difficult to make because the present study did not evaluate changes in senior or geriatric cats. It is unknown if the magnitude and duration of antinociception would have decreased in these cats as they become older.

Figure 2. Thermal nociceptive thresholds (°C) before (0 hour) and up to 12 hours after the intramuscular administration of saline 0.9% (0.05 mL/kg) or hydromorphone (0.1 mg/kg IM) in female cats of 6, 9, and 12 months of age. Data are presented as least square means ± SEM. * Significantly different when compared with baseline; / Significantly different between treatments.

Table 2

| Time point (h) | Age         | 6 months (n = 10) | 9 months (n = 10) | 12 months (n = 10) |
|---------------|-------------|------------------|------------------|-------------------|
| 0.25          | 3.6 ± 0.9   | 3.3 ± 0.9        | 4.1 ± 0.9        |
| 0.5           | 4.0 ± 0.9   | 2.5 ± 0.9        | 5.7 ± 0.9        |
| 0.75          | 4.0 ± 0.9   | 4.2 ± 0.9        | 6.8 ± 0.9        |
| 1             | 3.7 ± 0.9*  | 4.6 ± 0.9        | 7.2 ± 0.9*       |
| 2             | 2.9 ± 0.9*  | 3.6 ± 0.9        | 6.7 ± 0.9*       |
| 3             | 2.0 ± 0.9   | 3.4 ± 0.9        | 4.7 ± 0.9        |
| 4             | 1.6 ± 0.9   | 2.1 ± 0.9        | 3.1 ± 0.9        |

Delta was calculated as the difference between thermal thresholds at each time point minus the thermal threshold at baseline. Superscript letters indicate significant differences between age groups for the same time point.
5.3. Pharmacodynamics and hydromorphone-induced antinociception

Changes in the expression and physiology of opioid receptors, and pharmacodynamics with aging could explain at least in part differences in duration and magnitude of antinociception induced by hydromorphone in the present study. In human subjects, \( \mu \)-opioid receptor binding potential (B\(_{\text{max}}\)/K\(_{\text{d}}\)) in the neocortical and putamen regions in the brain increases with aging.\(^{53}\) In mice, receptor binding (B\(_{\text{max}}\)), receptor density, and total binding sites increase from postnatal to adult ages.\(^{2}\) This may contribute to reduced opioid thermal antinociception in pediatric subjects.\(^{47}\) Indeed, the number\(^{52}\) and density (2-fold)\(^{41}\) of opioid receptors increase from birth to adulthood in the rat brain. Additionally, the maturation of neural connections with the brain and descending inhibitory pathways may change with aging.\(^{2}\) For example, cells associated with the descending opioid inhibitory pathways located in the rostral ventral medulla and dorsal lateral pons require time to mature and to participate in opioid-induced antinociception.\(^{2}\)

5.4. Pharmacokinetics and hydromorphone-induced antinociception

Differences in antinociception could be due to pharmacokinetics. In cats, hepatic enzyme activity, plasma albumin concentrations, and glomerular filtration rates are similar to adult cats by 5 months of age.\(^{19,49}\) However, the volume of distribution is larger in young patients when compared with adults particularly with hydrophilic compounds (ie, low octanol/water partition coefficient) such as hydromorphone and morphine.\(^{3}\) Therefore, the administration of hydromorphone at a consistent dose would result in lower plasma concentrations in pediatric subjects when compared with adults because the amount of drug in a given volume of plasma depends on the amount of drug administered divided by the volume of distribution. This could explain the changes in duration and magnitude of antinociception with aging observed in this study.\(^{46}\) The short duration of action after hydromorphone could also be due to increased total body clearance in young/pediatric patients.\(^{9}\) Finally, treatments were administered by the intramuscular route, and it is possible that absorption was affected by aging due to physiological factors, such as lymph and blood flow, cardiac output, and the amount of fat distribution. These factors could influence the rate of absorption, drug bioavailability, and ultimately duration and magnitude of antinociception.\(^{45}\)

5.5. Adverse events

The incidences of vomiting, nausea, and dysphoria/euphoria observed in the present study are consistent with previous reports in cats administered with hydromorphone intramuscularly.\(^{36}\) Regardless of age, similar adverse events have been reported in human patients administered with opioids.\(^{10,35,37}\) Future studies should identify the effects of increased dosing frequency of hydromorphone in pediatric patients on the incidences of adverse events.

5.6. Limitations

This study possesses several limitations. Only female cats were evaluated. Data cannot be extrapolated for male individuals because previous studies report significant differences in the antinociceptive effects of opioids with aging.\(^{22}\) Sex may be associated with differences in opiate binding affinity at the supraspinal level.\(^{29,30}\) A second limitation is the predictive ability of thermal antinociception and its correlation to clinical analgesia. Despite the differences in nociceptive stimuli (clinical vs thermal), the duration of antinociception in the present study is similar to the duration of action when treating clinical pain in cats.\(^{4,33,42}\) Additionally, this study only evaluated a single dose of hydromorphone using a single route of administration. It is not known if results can be extrapolated for different dosage regimens. A pharmacokinetic–pharmacodynamic modeling of hydromorphone using different routes of administration and dosing in subjects at different ages could provide valuable insight on the antinociceptive effects of opioids and the findings of this study. Finally, senior and geriatric cats were not evaluated in this study, and it is unknown if the antinociceptive effects of hydromorphone would be different in this age group.

6. Conclusion

Hydromorphone provided a shorter duration and smaller magnitude of antinociception at 6 months when compared with 9 and 12 months of age in female cats. Pediatric cats may require more frequent dosing (ie, every 1–2 hours) of hydromorphone when compared with junior cats (adolescent) or prime adult cats (ie, every 3–4 hours). In this study, aging influenced onset, magnitude, and duration of opioid-induced feline thermal antinociception. This could have a clinical significance in terms of doses and intervals of opioid administration in pediatric vs adult patients.

### Table 3

| Age             | Treatment | Vomiting | | | Licking/Hypersalivation | | | Euphoria/Dysphoria | |
|-----------------|-----------|----------|---|---|------------------------|---|---|-------------------|
| 6 months (n = 10) | Saline    | 0        | 0.08 | 1 | 0.10 | 0 | 0.025 |
|                 | Hydromorphone | 3 | | 5 | | 5 | |
| 9 months (n = 10) | Saline    | 0        | 0.046* | 1 | 0.059 | 0 | 0.025* |
|                 | Hydromorphone | 4 | | 6 | | 5 | |
| 12 months (n = 10) | Saline    | 0        | 0.046* | 0 | 0.014* | 0 | 0.014* |
|                 | Hydromorphone | 4 | | 6 | | 6 | |

Data are presented as number of events. *P* value refers to the Mantel–Haenszel \( \chi^2 \) test for repeated measures.

* Significantly different between treatments in the prevalence of the adverse events.
Disclosures

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