Antinociceptive and anesthetic-sparing effects of levomethadone/fenpipramide cannot be enhanced by coadministration of metamizole in awake and anesthetized Beagles

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OBJECTIVE
To determine the effect of levomethadone/fenpipramide and metamizole alone and in combination on acute nociception.

METHODS
8 healthy, adult Beagles were used in 2 separate randomized, complete crossover, experimental trials (threshold testing and determination of minimal alveolar concentration [MAC]) with masked observers. In both trials, treatments were 0.2 mg·kg⁻¹ levomethadone/fenpipramide (L), 75 mg·kg⁻¹ metamizole (M), or their combination (LM). In conscious dogs, mechanical thresholds were determined using constantly rising force. Thermal thresholds were measured via ramped contact heat. The MAC of sevoflurane was determined using the bracketing method with electrical stimulus (50 V, 50 Hz, 10 ms) before and 1 and 4 hours after treatment.

RESULTS
Mechanical thresholds in L and LM were significantly increased above baseline (BL) for 165 minutes and above M for 135 minutes. Percent thermal threshold excursion significantly increased above BL in L for 75 minutes and in LM for 135 minutes. In L and LM, the percent thermal threshold excursion was significantly higher than in M from 15 to 75 or 135 minutes, respectively. In L and LM, the MAC of sevoflurane was significantly reduced at 1 hour compared to BL and M.

CONCLUSION
Duration but not the magnitude of thermal antinociception of levomethadone/fenpipramide was increased by metamizole. Mechanical antinociception in awake dogs and anesthetic-sparing effects of levomethadone/fenpipramide were not altered.

CLINICAL RELEVANCE
Coadministration of levomethadone/fenpipramide and metamizole to increase antinociception is not justified.

Keywords: minimal alveolar concentration (MAC), threshold, dipyrone, nociception, pain

Received April 19, 2024
Accepted July 26, 2024
Published online August 8, 2024
doi.org/10.2460/ajvr.24.04.0118

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American Journal of Veterinary Research
In laboratory Beagles, metamizole alone (50 mg·kg⁻¹) does not produce acute cutaneous antinociception or an anesthetic-sparing effect.²⁻⁵ However, in laboratory rodents, there is evidence that metamizole might influence descending pain pathways³⁻⁶ and the endogenous opioid system.¹¹⁻¹² The synergistic effects of the µ-opioid-receptor agonist morphine and metamizole have been shown in rodents. Therefore, a synergistic effects of µ-receptor agonist opioids and metamizole in dogs might be possible.

Hence, the aim of this study was to investigate if the antinociceptive action of levomethadone/fenpipramide in dogs can be enhanced by coadministration of metamizole. It was hypothesized that the antinociceptive action of levomethadone/fenpipramide will be enhanced by metamizole.

**Methods**

The study design was ethically reviewed and approved by the Lower Saxony State Office for Consumer Protection and Food Safety (LAVES), according to regulations of the German Animal Welfare Act (33.12 42502-04-12/0940).

The work was carried out as 2 separate experimental trials including the same dogs. The first part of the study evaluated the impact of levomethadone/fenpipramide, metamizole, or their combination on nociceptive thresholds in awake dogs, and the second part investigated their effect on the minimal alveolar concentration (MAC) of sevoflurane.

Sample size has been calculated (G*Power 3.1.9.2; Heinrich Heine University) based on the assumption to identify a clinically relevant difference of at least 20% between MAC values in the levomethadone and combination groups. Alpha and beta were set to 0.05 and 0.8, respectively. Calculations were based on an estimated MAC of sevoflurane of 2.39 ± 0.26%²⁰ and an estimated MAC reduction of 35% due to methadone.⁴ A sample size of 8 dogs was detected.

Drugs used in the present study are neither FDA approved nor commercially available in the US. Levomethadone/fenpipramide (L-Polamivet; MSD Tiergesundheit; Intervet Deutschland GmbH) is commercially available as a veterinary product in Germany. Metamizole (Novalgin Injektionslösung; Sanofi Aventis) is a commercially available human medicine product in Germany.

**Animals**

The same 8 laboratory Beagles (3 female spayed, 5 male neutered) were included in both parts of the study. Mean body weight was 15.9 ± 3.0 kg, and mean age was 3.7 ± 2.3 years. All dogs were regularly vaccinated and dewormed. They were judged to be healthy based on physical examination and full hematological and biochemical blood profile. The animals lived in groups of 4 to 6 dogs and were fed twice daily with a commercial dog diet (Science Plan Hills Canine Adult Medium, Advance Fitness; Hill’s Pet Nutrition GmbH), with water ad libitum and daily access to an enriched outdoor run.

**Study design**

The study was conducted as a prospective, randomized, experimental trial with complete crossover. The order of the drugs delivered was randomized by drawing prelabeled papers out of a bag. The observer was unaware of the treatment. Each dog went through both experimental protocols 3 times. According to the treatment group, dogs received 0.2 mg·kg⁻¹ levomethadone in fixed combination with fenpipramide (group L; L-Polamivet; MSD Tiergesundheit; Intervet Deutschland GmbH), 75 mg·kg⁻¹ metamizole without preservatives or solubilizers (group M; Novalgin Injektionslösung; Sanofi Aventis), or the combination of both (group LM) IV. With the use of sterile saline, drugs were diluted to a final volume of 5 mL and administered by a person not involved in threshold or MAC determination. A washout period of at least 14 days was respected between treatments.

On all study days, an IV catheter for drug administration was aseptically placed in 1 lateral saphenous vein. The methods used for evaluating thermal threshold (TT) and mechanical threshold (MT) as well as MAC are explained in detail elsewhere and will only be described in brief.

**Nociceptive threshold determination**

Nociceptive thresholds were assessed using manually operated, commercially available systems, which are evaluated for dogs (TT1 and MT1; Topcat Metrology Ltd). The MT was tested with a probe containing 3 blunt metal pins (diameter of 2.5 mm) placed over the dorsal aspect of radius and ulna of 1 limb. A nonfunctional dummy device was positioned on the opposite leg. The force was increased at a constant rate of 0.8 N/s. For TT measurement, a thermistor probe was placed over a clipped area on the lateral thoracic wall and secured with elastic ribbons. Constant skin contact was assured by inflating an air-filled bladder above the probe until the pressure reached values between 3.99 and 10.67 kPa (30 to 80 mm Hg). The rate of temperature rise was 0.6 °C each second (°C·s⁻¹). Force and temperature were increased until the dog showed a predefined purposeful reaction (looking at the device, biting into the probe, avoidance behavior, vocalization) or the safety cutout values (20 N; 55 °C). were reached. Baseline MT and TT were calculated as the mean of three consecutive measurements over 90 minutes before drug application. The test drug was injected as an IV bolus, with an injection speed of 1 mL·min⁻¹. After treatment, MT and TT were determined at the time points of 15, 45, 75, 105, 135, 165, 195, 225, 255, 285, 315, 345, 375, 435, and 495 minutes after drug injection. The selection of time points was based on data from Hoffmann et al., documenting a maximal TT after 45 minutes and maximal plasma.
concentrations 75 minutes after IM levomethadon/ fenpipramide application. Utilizing IV drug application an earlier drug effect was anticipated. Starting at 15 minutes and reevaluating every 30 minutes allowed measurements at an early point and at the times (45 and 75 minutes) when Hoffmann et al. documented the largest changes. Mechanical and thermal tests for each dog were run directly after each other in alternating order to prevent conditioning and learning effects. Furthermore, occasional mock tests with the probes not connected to the control device were interspersed. After the last measurements, probes were removed and the dogs were examined for signs of skin irritation, burns, or injury.

For preclusion of possible influences of variable skin temperatures at the start point of the TT measurement, the percent TT excursion (%TE) was calculated. Therefore, the TT temperature, the skin temperature (TS), and the thermal cutout temperature (TC) of 55 °C were used in the following equation:

\[ \%TE = 100 \times \frac{(TT - TS)}{(TC - TS)} \]

**Minimal alveolar concentration determination**

**Anesthesia induction and instrumentation**

Anesthesia was induced by a facemask and maintained with sevoflurane (SevoFlo; Abbott Laboratories Ltd) in oxygen after endotracheal intubation. Dogs were positioned on a heating pad, and volume-controlled continuous mandatory ventilation was initiated to maintain normocapnia (Paco\(_2\); 4.66 to 5.99 kPa; 35 to 45 mm Hg). Body temperature, ECG, heart rate from ECG, breathing frequency, oxygen saturation (SpO\(_2\)), inspiratory and end-tidal oxygen, carbon dioxide, and sevoflurane concentrations were measured continuously by a multiparameter anesthesia monitor (Compact Anesthesia Monitor; Datex-Ohmeda; GE Healthcare) and recorded every 5 minutes. Gases were sampled via coaxial tubing at the distal end of the endotracheal tube, and gas concentrations were determined by side stream technique using infrared light absorption. The gas analyzer was calibrated before each experiment. The site where the experiments were performed was 55 m above sea level, and MAC values were not corrected to sea level. An arterial catheter was placed in the dorsal pedal artery for direct blood pressure measurement, blood gas analysis, and evaluation of pulse rate.

For nociceptive stimulation, 2 electrodes (TECA disposable monopolar needle electrode; 50 mm, 26G; Viasys Healthcare) were placed SC at the medial aspect of the left thoracic limb in the region of radius and ulna. The electrical stimulation included 2 single and 2 continuous stimuli each separated by 5 seconds generated by a square pulse generator (Grass S48 Square Pulse Stimulator; Natus Neurology Incorporated). The defined electrical stimulus (50 V at 50 cycles·s\(^{-1}\) for 10 milliseconds) was ceased as soon as a positive response was visible or the end of the cycle was reached. Gross purposeful movements (lifting of the head, motion of the hind limbs, or the tail) were considered a positive reaction. After instrumentation, dogs were covered with a warm air filled blanket to maintain body temperature between 37 and 38.5 °C.

Dogs received a constant rate infusion of a balanced electrolyte solution (Sterofundin; B. Braun Melsungen AG) at 5 mL·kg\(^{-1}\)·h\(^{-1}\). In the case of mean arterial blood pressure (MAP) falling below 60 mm Hg, a fluid bolus of 20 mL·kg\(^{-1}\) was administered over 10 minutes. If this could not successfully increase MAP, a dopamine constant rate infusion (dopamine-ratiopharm at 50 µg·mL\(^{-1}\); ratiopharm GmbH) was started with 3 µg·kg\(^{-1}\)·min\(^{-1}\). The infusion rate was stepwise increased by 1 µg·kg\(^{-1}\)·min\(^{-1}\) every 5 minutes to a maximum of 10 µg·kg\(^{-1}\)·min\(^{-1}\) until a MAP above 60 mm Hg was reached.

**Determination of MAC values**

Before each stimulation for MAC evaluation, the end-expiratory sevoflurane concentration was kept constant for at least 20 minutes. Stimulation was started at 2.3 vol% sevoflurane, which is documented as the sevoflurane MAC of dogs. Following each nociceptive stimulation, sevoflurane concentration was either increased (positive reaction/purposeful movement) or decreased (negative reaction/no purposeful movement) by 0.1 vol%. The MAC was calculated as the mean value of the end-expiratory concentration just preventing purposeful movement and the one just allowing purposeful movement. After identification of baseline MAC, drugs were applied, and MAC was reevaluated 1 and 4 hours later. All animals recovered in a silent, warm kennel and received 4 mg·kg\(^{-1}\) carprofen, IV (Rimadyl Injektionslösung; Pfizer GmbH).

**Statistical analysis—**Statistical data analysis was executed using the software GraphPad Prism 4 (GraphPad).

Data were tested for normal distribution using the Shapiro-Wilk test.

For parametric data, intratreatment differences were evaluated by one-way ANOVA for repeated measurements with Bonferroni post hoc tests. Intergroup differences for MT were investigated by 2-way ANOVA for repeated measurements followed by Bonferroni post hoc test where appropriate. Due to 3 missing values, intergroup differences for percent TT excursion were investigated by a mixed effect analysis for repeated measures followed by the Tukey multicomparison test where appropriate.

The level of significance was set to be less than 5%.

**Results**

**Nociceptive thresholds**

Compared to baseline, MT was significantly increased over 165 minutes in L and LM (\(P \leq 0.0284\)). Cutout values were reached from 75 minutes (L, 8/8 dogs; LM, 7/8) until 135 minutes (L, 4/8; LM, 4/8). Baseline MT was attained again after 375 and
465 minutes in L and LM, respectively. In M, no significant differences to baseline occurred.

The MT was significantly higher in L \((P \leq 0.0021)\) and LM \((P \leq 0.0017)\) than in M from measurement points 15 to 135 minutes. Treatment L and LM were not different for MT (Figure 1).

Skin temperature stayed above 34 °C in all treatments over the whole study period.

Percent TT excursion was significantly increased over baseline for 75 minutes in L \((P \leq 0.0118)\) and for 135 minutes in LM \((P \leq 0.0089)\). In M, percent TT excursion was not altered compared to baseline.

Compared to M, percent TT excursion was significantly higher in L from 15 to 75 minutes \((P \leq 0.0003)\) and in LM from 15 to 135 minutes \((P \leq 0.0075)\). In LM, percent TT excursion was significantly higher than in L at time points 105 and 135 minutes \((P \leq 0.045; \text{Figure 2})\).

Minimal alveolar concentration

Results for MAC evaluation are shown (Table 1). In 3 different dogs (1 in each treatment group) a bolus of crystalloid solution and dopamine constant rate infusion at 5 \(\mu \text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\) were necessary to keep MAP above 60 mm Hg. Total anesthesia time (time from endotracheal intubation to end of sevoflurane administration; L, 418 min; LM, 399 min; and M, 387 min) was not different between treatments.

<table>
<thead>
<tr>
<th></th>
<th>M</th>
<th>L</th>
<th>LM</th>
</tr>
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<tbody>
<tr>
<td>BL</td>
<td>2.5 ± 0.3</td>
<td>2.6 ± 0.5</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>1 hour</td>
<td>2.5 ± 0.4</td>
<td>1.3 ± 0.4\textsuperscript{a,b}</td>
<td>1.4 ± 0.5\textsuperscript{a,b}</td>
</tr>
<tr>
<td>4 hours</td>
<td>2.4 ± 0.3</td>
<td>2.2 ± 0.4</td>
<td>2.2 ± 0.5</td>
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\textsuperscript{a,b}Significant differences to BL values within the treatment group. \textsuperscript{a}Significant differences to group M at the same time point.

Discussion

In the present trial, the acute antinoceptive action of levomethadone/fenpipramide in awake and anesthetized dogs could not be enhanced by coadministration of metamizole. Despite a minor prolongation of the effect on TT, the hypothesis must be rejected.

This study indicates that levomethadone/fenpipramide and metamizole might not have a positive interaction concerning the magnitude of antinoception in the dog. In contrast to this, synergistic effects of morphine and metamizole are demonstrated in laboratory rodents.\textsuperscript{18,19} A potential
reason for this disparity could be the use of different opioid drugs or the difference in the test model. The present study used acute nociceptive threshold evaluation with short-lasting stimuli mainly exciting Aβ fibers. In contrast, Aguirre-Bañuelos and Granados-Soto\textsuperscript{18} used the formalin test, which causes local tissue damage and inflammation with a bipartite response involving mainly C fibers and nocinplastic changes in the dorsal horn of the spinal cord. Furthermore, laboratory studies showing the interaction of metamizole with the endogenous opioid system used direct (micro) injection to distinct parts of the CNS.\textsuperscript{12,22} It might be that with direct local application into the CNS or the paw higher drug concentrations at the effect site were achieved than after IV administration.

In addition, using safety cutouts for ethical and animal safety reasons could have mitigated existing differences between groups L and LM concerning nociceptive thresholds in the present trial. In MAC determination, measurement times and repetitions are limited due to the time-consuming technique; therefore, it is not known if stimulations at other time points would have demonstrated differences.

In the present trial, 2 established methods (MAC and nociceptive thresholds) were used as surrogates to experimentally assess intra- and postoperative nociception. For classical NSAIDs, a MAC reduction of up to 10% in dogs is documented.\textsuperscript{24} In contrast, both 50\%\textsuperscript{17} and 75 mg·kg\textsuperscript{−1} metamizole could not demonstrate a MAC-sparing effect. Also, the combination of metamizole and levomethadone/fenpipramide did not lead to a significant difference in MAC values between groups L and LM. After levomethadone/fenpipramide, a significant decrease in sevoflurane requirements of over 40% could be detected for both groups (L and LM), which agrees with the documented effect of methadone on isoflu-rane MAC in dogs.\textsuperscript{4} However, a decrease in inhalant requirements (MAC) cannot clearly be attributed to antinociception, as MAC is a measurement of spinally mediated immobility which can be influenced by mesencephalic centers.\textsuperscript{26} Therefore, also drugs causing hypnosis or immobility could lead to a reduction in MAC value.\textsuperscript{27} To differentiate between true antino-ciception and possible centrally depressant effects, MAC determination was combined with TT and MT determination as classic nociceptive tests for evaluation of drug effects in the conscious dog.

Both tests utilized in the present study were able to detect the action of μ-receptor agonist opioids but have been proven to remain unchanged after 50 mg·kg\textsuperscript{−1} metamizole alone.\textsuperscript{17} Even a 50% increase in metamizole dose to 75 mg·kg\textsuperscript{−1}, a higher than clinically used dose, did not produce detectable antino-ciception after metamizole in this trial. Therefore, it might be that these techniques/models are not sensitive enough to detect a possible synergistic or agonistic effect of levomethadone and metamizole. To assess the action of classical NSAIDs, tests inducing inflammation before antinociceptive testing are used.\textsuperscript{28} In one of these tests, the carageen test, pain reduction in rats after metamizole is documented.\textsuperscript{29} Therefore, this or other tests, for example, visceral stimulation, could have shown different results. Nevertheless, the aim was to use an experimental setup mimicking surgically induced nociceptive stimulation. Therefore, results cannot be transferred to situations when other types of pain like inflammatory or neuropathic pain are prevalent. For example, it is known that metamizole can contribute to sufficient postoperative analgesia in dogs receiving methadone for intraoperative analgesia.\textsuperscript{30}

Elevation over baseline of TTs but not MTs in group LM was significantly longer than in group L. The reason for this observation is not clear. During coadministration with fluconazole increased metha-done plasma levels and a longer elevation of plasma levels than compared to methadone alone could be demonstrated.\textsuperscript{31} Interaction of the coadministered drug with cytochrome P450 enzymes leading to changed metabolism were discussed as possible reasons for the increased plasma levels. Metabolites of metamizole interact with cytochrome (CYP) 3A4, CYP 2B6, CYP 2C8, and 2C9 enzymes in humans.\textsuperscript{32} However, induction but not inhibition of CYP 2B6 and CYP 3A4 by metamizole is documented in a model using human hepatocytes.\textsuperscript{33} Presuming this would be equal in dogs faster metabolism could be anticipated, which cannot explain the findings of the present study. To the authors’ knowledge, no data about the influence of metamizole or it is metabolites on canine cytochrome P450 are available. Furthermore, this trial studied 1 bolus injection of drugs, and enzyme induction or inhibition is usually observed during medium to long-term treatments. At present, no pharmacological studies about the combination of (levo)methadone and metamizole in dogs are available. Therefore, discussion about possibly changed pharmacokinetics of methadone and factors that could be involved (eg, plasma-protein binding) would remain highly speculative. Furthermore, it is questionable if the statistical difference in the thermal data seen between groups L and LM at 2 measurement points exhibits clinical importance, especially as the MT and MAC evaluation did not reveal any differences between groups L and LM.

The present trial was an experimental study within a small and homogenous population using predefined stimuli and drug doses. This might limit transferability to a clinical situation. Furthermore, a model using acute antinociception does not comprehensively picture complex clinical pain but rather can be seen as a preclinical test.

In conclusion, the antinociceptive effect of levomethadone/fenpipramide in awake or anesthetized Beagles could not be enhanced by coadministration of metamizole. Therefore, the coadministration of these drugs to increase antinociception is not justified. Conclusions and recommendations about the usage of the drug combination for other types of pain cannot be drawn.

**Acknowledgments**

None reported.
Disclosures

Dr. Kästner is a member of the AJVR Scientific Review Board but was not involved in the editorial evaluation of or decision to accept this article for publication.

No AI-assisted technologies were used in the generation of this manuscript.

Funding

The authors acknowledge financial support by the Open Access Publication Fund of the University of Veterinary Medicine Hannover, Foundation.

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