Antihistaminic and cardiorespiratory effects of diphenhydramine hydrochloride in anesthetized dogs undergoing excision of mast cell tumors

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OBJECTIVE
To evaluate the effects of IV diphenhydramine hydrochloride administration on cardiorespiratory variables in anesthetized dogs undergoing mast cell tumor (MCT) excision.

DESIGN
Randomized, blinded clinical trial.

ANIMALS
16 client-owned dogs with MCTs.

PROCEDURES
In a standardized isoflurane anesthesia session that included mechanical ventilation, dogs received diphenhydramine hydrochloride (1 mg/kg [0.45 mg/lb], IV; n = 8) or an equivalent volume of saline (0.9% NaCl) solution (IV; control treatment; 8) 10 minutes after induction. Cardiorespiratory variables were recorded throughout anesthesia and MCT excision, and blood samples for determination of plasma diphenhydramine and histamine concentrations were collected prior to premedication (baseline), throughout anesthesia, and 2 hours after extubation.

RESULTS
Cardiorespiratory values in both treatment groups were acceptable for anesthetized dogs. Mean ± SD diastolic arterial blood pressure was significantly lower in the diphenhydramine versus control group during tumor dissection (52 ± 10 mm Hg vs 62 ± 9 mm Hg) and surgical closure (51 ± 10 mm Hg vs 65 ± 9 mm Hg). Mean arterial blood pressure was significantly lower in the diphenhydramine versus control group during surgical closure (65 ± 12 mm Hg vs 78 ± 11 mm Hg), despite a higher cardiac index value. Plasma histamine concentrations were nonsignificantly higher than baseline during maximal manipulation of the tumor and surgical preparation in the diphenhydramine group and during surgical dissection in the control group.

CONCLUSIONS AND CLINICAL RELEVANCE
IV administration of diphenhydramine prior to MCT excision had no clear clinical cardiorespiratory benefits over placebo in isoflurane-anesthetized dogs. (J Am Vet Med Assoc 2017;251:804–813)

Mast cell tumor is the most commonly diagnosed malignant skin tumor in dogs. Cutaneous mastocytoma is the most common type of MCT and originates from mast cells in the dermis and subcutaneous tissue; however, MCT can potentially originate from any location where mast cells are found, including the lungs, intestinal mucosa, and perivascular regions. Paraneoplastic syndromes associated with MCT in dogs are usually the result of mast cell degranulation of bioactive substances, such as histamine, heparin, proteolytic enzymes, and vasoactive amines, precipitated by physical or chemical means, including surgical manipulation of the tumor or radiation therapy.

In dogs, systemic release of histamine causes dilation of arteries and terminal arterioles and increases capillary permeability through actions on H1 and H2 receptors, leading to a decrease in systemic vascular resistance and arterial blood pressure. Direct effects of histamine on heart function in dogs include predominant activation of H1 receptors, which may cause a decrease in ventricular contractility and may impair atrioventricular conduction leading to atrioventricular dissociation. These adverse cardiovascular effects are dose related and may be short lived owing...
to compensatory reflexes and quick inactivation of histamine.\textsuperscript{11,12} Effects of anesthetic drugs or hemodynamic depression may blunt sympathetic nervous responses and interfere with compensatory responses to histamine-induced vasodilation, which may lead to dramatic hypotension or even vascular collapse.\textsuperscript{11}

Histamine release is presumed to occur as a result of degranulation from MCTs during surgical manipulation, but no information is available regarding changes in circulating amounts of histamine that may occur in anesthetized dogs undergoing MCT excision. Preemptive administration of diphenhydramine to dogs undergoing MCT excision has been recommended to prevent potential adverse cardiovascular effects of histamine,\textsuperscript{13} although this type of preventive treatment is not instituted universally. In addition, neither therapeutic doses for dogs nor the effectiveness of diphenhydramine for preventing cardiovascular histamine-related responses have been established in this clinical scenario.

Diphenhydramine is a first-generation inverse agonist of H\textsubscript{1} receptors that binds to and stabilizes the inactive conformation of H\textsubscript{1} receptors,\textsuperscript{14} preventing the effects of histamine. Administration of diphenhydramine at 1 mg/kg (0.45 mg/lb), IV, to healthy conscious dogs results in rapid and high plasma diphenhydramine concentrations without affecting cardiorespiratory function and for a duration that could exceed the surgical time required for MCT excision.\textsuperscript{15} Research in anesthetized dogs and cats has shown that diphenhydramine can abolish the vasodilatory effect of low doses of histamine;\textsuperscript{16} however, diphenhydramine and other H\textsubscript{1}-receptor antihistamines administered are reportedly unable to abolish the severe cardiovascular depression caused by high doses of exogenous histamine when administered alone.\textsuperscript{17–18}

The purpose of the study reported here was to determine the possible therapeutic effects of diphenhydramine hydrochloride (1 mg/kg, IV) in dogs anesthetized for MCT excision by investigating anesthetic stability, cardiorespiratory responses, and incidence of hypotension with or without diphenhydramine administration in such dogs and relationships of anesthesia and cardiorespiratory variables with plasma histamine and diphenhydramine concentrations during those events. The hypothesis was that plasma histamine concentrations would affect arterial blood pressure in isoflurane-anesthetized dogs and that preoperative diphenhydramine administration would minimize any variations in blood pressure associated with changes in plasma histamine concentration during surgical manipulation of MCTs.

Materials and Methods

Animals

Client-owned dogs admitted to the Ontario Veterinary College Health Sciences Centre between August 2014 and March 2015 for MCT excision requiring general anesthesia were eligible for inclusion in the study. Dogs were enrolled if they had a cytologic or histologic diagnosis of MCT and weighed > 10 kg (22 lb). Dogs with clinically important cardiovascular disease (receiving heart medication) or renal disease (azotemia) were excluded.

Sixteen dogs (11 females and 5 males) met the inclusion criteria. Dogs were classified as Pug (n = 3), mixed breed (3), Boxer (2), Labrador Retriever (2), Golden Retriever (2), Boston Terrier (1), Rottweiler (1), Weimaraner (1), and Beagle (1). Body weight ranged from 10 to 35 kg (22 to 77 lb) and age ranged from 4 to 14 years. For dogs that were already receiving diphenhydramine or other antihistamines preoperatively, treatment was stopped if at all possible at least 12 hours prior to anesthesia.

The study was carried out in accordance with the guidelines of the Canadian Council on Animal Care and was approved by the Institutional Animal Care Committee at the University of Guelph. Owner consent was obtained in writing for all dogs prior to anesthesia.

Study protocol

A CBC and serum biochemical analysis were performed for each dog prior to anesthesia. A physical examination of each dog was performed prior to premedication by the same anesthesiologist (AS), and rectal temperature, HR, and respiratory rate were recorded. A blood sample (6 mL) was collected by jugular venipuncture for baseline determination of plasma diphenhydramine (measurement point D1) and histamine (H1) concentrations. Immediately afterward, hydromorphone hydrochloride\textsuperscript{a} (0.1 mg/kg) was administered IM.

Depending on the location of the MCT, a cephalic or saphenous vein was catheterized (20- or 22-gauge 1- to 1.88-inch catheter\textsuperscript{b}). Anesthesia was induced with propofol\textsuperscript{c} (2 to 4 mg/kg [0.9 to 1.8 mg/lb], IV) to effect. Dogs were then endotracheally intubated, and anesthesia was maintained with isoflurane\textsuperscript{d} in oxygen by use of a coaxial rebreathing system (F-circuit) with an O\textsubscript{2} flow rate of 50 to 100 mL/kg/min (23 to 45 mL/lb/min). To maintain end-tidal CO\textsubscript{2} values between 30 and 40 mm Hg, intermittent positive pressure ventilation was immediately provided by means of an electronically controlled, time-constant pressure-limited ventilator,\textsuperscript{e} with a rate of 8 to 12 breaths/min and a tidal volume of 10 to 15 mL/kg (4.5 to 6.8 mL/lb). Dogs were instrumented during the first 5 to 10 minutes of anesthesia, and the isoflurane vaporizer setting was adjusted to maintain a constant end-tidal isoflurane concentration of 1.5%. A balanced electrolyte solution was administered at a rate of 10 mL/kg/h throughout anesthesia.

A dorsal pedal artery was catheterized with a 20- or 22-gauge 1- to 1.88-inch catheter\textsuperscript{f} for arterial blood pressure monitoring, blood gas analysis, and blood sample collection for determination of plasma diphenhydramine and histamine concentrations during anesthesia. This arterial catheter was connected by noncompliant tubing to a pressure transducer\textsuperscript{g}.
from a multiparametric anesthesia monitor for determination of SAP, DAP, and MAP. The transducer was verified against a mercury manometer at 50, 100, and 200 mm Hg. The monitor was zeroed prior to blood pressure determinations, and the transducer was placed at the level of the manubrium. The same multiparameter monitor was used to monitor HR, respiratory rate, end-tidal isoflurane concentration, and esophageal temperature. A noninvasive cardiac output with partial rebreathing of CO₂ and esophageal temperature. A noninvasive cardiac output with partial rebreathing of CO₂ monitor was used for measurement of CO₂ monitor was used for measurement of cardiac output, SpO₂, and end-tidal CO₂ concentration. During the instrumentation phase, an arterial blood sample was obtained from the dorsal pedal artery for arterial blood gas analysis. Values of Pao2, Paco2, blood hemoglobin concentration, and fraction of inspired O₂ obtained from the arterial blood gas analysis were entered into the cardiac output monitor. This process was repeated at the beginning of the surgical procedure to ensure accurate cardiac output measurements due to changes in blood gas parameters during anesthesia. The cardiac index value was calculated by dividing the obtained cardiac output value by the dog's body weight, and SVI was calculated as (cardiac output / HR) / body weight.

Dogs were then randomly assigned to 2 treatment groups (diphenhydramine or control group) by use of an online randomization scheme. After instrumentation and recording of baseline cardiorespiratory variables, treatments were administered. Dogs in the diphenhydramine group received diphenhydramine hydrochloride (1 mg/kg, IV), and dogs in the control group received an equivalent volume of saline (0.9% NaCl) solution, IV.

Cefazolin (22 mg/kg [10 mg/lb], IV) was administered to all dogs after anesthetic induction and then every 90 minutes until the end of surgery. A recirculating warm water blanket and delivery of forced warm air were used to maintain esophageal body temperature within reference limits.

A board-certified surgeon performed excision (marginal or wide) of the tumor. After the surgical procedure, dogs were transferred to a recovery area and isoflurane administration was discontinued. Following extubation, dogs were kept in a cage with the arterial and venous catheters in place and monitored until fully recovered. At 2 hours after extubation, a blood sample was collected from the arterial catheter for measurement of plasma diphenhydramine and histamine concentrations. Additional doses of hydrocortisone (0.025 mg/kg [0.011 mg/lb], IV) were administered 2 to 3 hours after the premedication dose throughout surgery and thereafter if signs of pain were noticed in the postextubation period.

For all dogs, tissues obtained during surgery were sent for histologic confirmation of the diagnosis and grading of the tumor. The World Health Organization Clinical Staging System was used for staging, and 2-tier grading criteria were used to grade cutaneous tumors, but not oral or subcutaneous tumors, given that there is no universally accepted available grading system for tumors at these other sites.

### Monitoring and blood sample collection

Cardiorespiratory variables (HR, SAP, MAP, DAP, cardiac index, SVI, end-tidal isoflurane concentration, tidal volume, respiratory rate, end-tidal CO₂ concentration, and SpO₂) and esophageal temperature were monitored continuously after anesthetic induction and throughout anesthesia. These values were recorded immediately before aseptic preparation of the surgical site (T0); 5 minutes after treatment administration (T1); 10 minutes after treatment administration, during aseptic preparation of the surgical site (T2); 20 to 30 minutes after treatment administration (T3); 30 to 40 minutes after treatment administration (T4); within 10 minutes after draping the surgical site (T5); halfway through excision of the tumor (T6); immediately before complete excision of the tumor (T7); and halfway through closure of the excision site (T8: Figure 1).

Hypotension during anesthesia was defined as MAP < 60 mm Hg and was treated with dopamine (5 µg/kg/min [2.3 µg/lb/min], IV) if it persisted for > 10 minutes or was deemed detrimental to the patient. The same investigator (AS), who was blinded to the treatment received, performed all anesthetic procedures, including blood sample collection, monitoring of anesthetic depth, and recording of anesthetic parameters.

Figure 1—Timeline for dogs anesthetized for MCT excision that received diphenhydramine hydrochloride (1 mg/kg [0.45 mg/lb], IV; n = 8) or an equivalent volume of saline (0.9% NaCl) solution (IV; 8) after induction and in which plasma histamine (H) and diphenhydramine (D) concentrations and cardiorespiratory variables were measured before premedication (H1, D1, or PRE, respectively) and at various points afterward. Measurement times for cardiorespiratory variables after premedication included immediately before aseptic preparation of the surgical site (T0); 5 minutes after treatment administration (T1); 10 minutes after treatment administration, during aseptic preparation of the surgical site (T2); 20 to 30 minutes after treatment administration (T3); 30 to 40 minutes after treatment administration (T4); within 10 minutes after draping the surgical site (T5); halfway through excision of the tumor (T6); immediately before complete excision of the tumor (T7); and halfway through closure of the excision site (T8).
In addition to blood samples collected for measurement of baseline plasma histamine (H1) and diphenhydramine (D1) concentrations, 3 mL of blood was collected from the arterial catheter for each measurement of these concentrations during and after surgery (Figure 1). Samples were placed on ice immediately after collection until centrifugation at 3,000 X g for 10 minutes. Plasma was then harvested, plasma samples for histamine analysis were placed in EDTA tubes, plasma samples for diphenhydramine analysis were placed in heparinized tubes, and both sets of tubes were stored at –80°C until analysis. Additional collection points for measurement of plasma histamine concentration were after induction, at T0 (H2); during maximal manipulation of the tumor at the time of surgical preparation, at T3 or T4 (H3); during surgery just before tumor removal, at T7 (H4); and 2 hours after extubation, at T9 (H5). Additional collection points for measurement of plasma diphenhydramine concentration were 5 minutes after treatment administration, at T1 (D2); 30 minutes after treatment administration, at T3 or T4 (D3); during surgical dissection of the tumor, at T6 (D4); just before tumor removal, at T7 (D5); and 2 hours after extubation, at T9 (D6). The volume of blood removed after each sample collection was replaced through the arterial catheter with twice the volume of saline solution at each measurement point.

**Plasma diphenhydramine analysis**

Plasma diphenhydramine concentration was measured by means of high-performance liquid chromatography with fluorescence detection as described elsewhere. Specialized software was used for data collection and processing. The limit of detection for this assay was 5 ng/mL, and the limit of quantification was 10 ng/mL. Intraday and interday assay coefficients of variation were 6.9% and 13.7%, respectively.

**Plasma histamine analysis**

Plasma histamine concentration was measured by use of a commercial enzyme immunoassay kit in accordance with the manufacturer’s recommendations. This assay has a limit of quantification of 0.055 ng/mL (0.5nM) and has been validated and used for determination of plasma histamine concentrations in dogs. The assay has an intra-assay precision of 7.1% to 9.8% and interassay precision of 6.4% to 16.8%.

**Statistical analysis**

Continuous data were tested for normality of distribution by means of the Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling tests. Data were also assessed for unequal variances, outliers, and other nonrandom patterns. Effects of treatment on cardiorespiratory variables were determined by creation of a generalized mixed linear model that allowed for correlations and nonconstant variability for normally distributed data or nonparametric data. The error structure over time was selected on the basis of the optimal Akaike information criterion value achieved with the structures provided offered by the statistical software, using for each treatment a random effect and a 2-factor (time and treatment) factorial design with repeated measurements.

Normal distribution of plasma diphenhydramine concentrations was evaluated with the Kolmogorov-Smirnov test followed by computation of descriptive statistics to assess symmetry and confidence intervals. Differences among measurement points were assessed through repeated-measures ANOVA followed by post hoc Bonferroni correction for comparisons between the 2 treatment groups. Differences in sex, age, body weight, and propofol dose were evaluated with the paired-samples *t* test.

Plasma diphenhydramine concentrations within the diphenhydramine group and those within and between groups were compared by use of repeated-measures ANOVA followed by post hoc Bonferroni correction. Normality of data distribution was assessed with the Kolmogorov-Smirnov test.

Values are reported as mean ± SD for normally distributed data or median (95% confidence interval) for nonnormally distributed data. Values of *P* < 0.05 were considered significant for all analyses.

**Results**

**Animals**

Histologic examination revealed that 8 dogs had subcutaneous MCTs and the other 8 had cutaneous MCTs. Of the 8 dogs with cutaneous MCTs, 4 tumors were classified as high grade. Seven dogs (3 in the diphenhydramine group and 4 in the control group) were in stage I of the disease, 4 dogs (2 in each group) were in stage II, 4 dogs (2 in each group) were in stage III, and 1 dog in the diphenhydramine group had systemic disease with splenic involvement and was considered to be in clinical stage IV.

No differences were identified between groups in dog characteristics. The diphenhydramine group contained 5 males and 3 females (*P* = 1.00). Mean ± SD body weight in the diphenhydramine group was 25.4 ± 12.2 kg (55.9 ± 26.8 lb) and in the control group was 26.9 ± 9.7 kg (59.2 ± 21.3 lb); mean age was 8.1 ± 3.5 years and 8.0 ± 2.1 years, respectively; and mean propofol dose required for anesthetic induction was 3.0 ± 0.8 mg/kg (1.4 ± 0.4 mg/lb) and 3.5 ± 0.9 mg/kg (1.6 ± 0.4 mg/lb), respectively. Induction of anesthesia occurred a mean of 42 ± 23 minutes after premedication, and treatments were administered a mean of 11 ± 7 minutes after anesthetic induction.

**Anesthesia and cardiorespiratory variables**

No significant differences were identified between the diphenhydramine and control groups in esophageal temperature (mean ± SD, 36.6 ± 0.8°C [97.9 ± 1.4°F] and 36.9 ± 0.7°C [98.4 ± 1.3°F], re-
respectively), end-tidal isoflurane concentration (1.5 ± 0.1% and 1.4 ± 0.1%, respectively), $\text{SpO}_2$ (100 ± 0.7% and 99 ± 0.7%, respectively), $\text{Pao}_2$ (408 ± 89 mm Hg and 473 ± 80 mm Hg, respectively), or $\text{Paco}_2$ (41 ± 5 mm Hg and 40 ± 5 mm Hg, respectively) at any measurement point.

No significant differences were identified between treatment groups in SVI, HR, and SAP at any measurement point (Table 1). Heart rate decreased after premedication with hydromorphone in both groups. In the diphenhydramine group, HR was significantly ($P < 0.001$) lower at T0 than before premedication; in the control group, the decrease in HR from that at T0 was significant ($P = 0.046$) after anesthetic induction (T1). No significant changes in any measured cardiovascular variable were detected within the diphenhydramine group between baseline (T0) and 5 minutes after treatment administration (T1).

In the control group, SAP was significantly lower than T0 at T2 ($P = 0.03$), T3 ($P = 0.03$), T4 ($P = 0.02$), and T5 ($P = 0.008$), whereas in the diphenhydramine group, it was lower than T0 at T3 ($P = 0.04$), T4 (0.02), T7 ($P = 0.02$), and T8 ($P = 0.04$; Figure 2). Values for DAP and MAP were lower than T0 at T4 in the diphenhydramine group ($P = 0.046$ for DAP and $P = 0.02$ for MAP). Mean MAP and DAP increased significantly at the time of surgery (T6, T7, and T8) with respect to values at T0 in the control group ($P = 0.04$ at T6, $P = 0.004$ at T7, and $P < 0.001$ at T8 for MAP; $P = 0.007$ at T6, $P < 0.001$ at T7, and $P < 0.001$ at T8 for DAP). At T7 ($P = 0.02$) and T8 ($P = 0.05$), MAP was significantly greater in the control group versus the diphenhydramine group, as was DAP at T6 ($P = 0.049$), T7 ($P = 0.007$), and T8 ($P = 0.007$).

Cardiac index values were significantly ($P = 0.03$) lower than at T0 at T3 in the control group (Figure 2). A clinical increase in cardiac index values was identified in both groups and an increase in SVI identified in the diphenhydramine group during surgery with respect to previous values, although these changes were not significant. Cardiac index values were significantly ($P = 0.047$) higher in the diphenhydramine group at the time of complete excision of the tumor (T7) than in the control group. Surgical stimulation also resulted in significant increases in HR ($P = 0.002$), DAP ($P = 0.008$), and MAP ($P = 0.005$) between T5 and T6 in the control group but not in the diphenhydramine group.

Four dogs in each group developed transient hypotension (MAP range, 44 to 59 mm Hg) between T1 and T5, but dopamine administration was only required for 2 of these dogs owing to hypotension that persisted for >10 minutes. One dog was from the control group and required dopamine administration for 7 minutes, and the other dog was from the diphenhydramine group and required dopamine administration time for 75 minutes.

**Plasma diphenhydramine and histamine concentrations**

No differences between the diphenhydramine and control groups were detected in timings of blood sample collection for plasma diphenhydramine or histamine concentration assays. Only 1

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**Table 1**—Mean ± SD values of cardiovascular variables and measurement timings at various points for dogs anesthetized for MCT excision that received diphenhydramine (1 mg/kg [0.45 mg/lb]; IV; n = 8) or an equivalent volume of saline (0.9%) solution (IV; control group; 8) after induction.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PRE</th>
<th>T0</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
<th>T8</th>
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<tr>
<td>Time before or after treatment (min)</td>
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<td>5 ± 0</td>
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<td>24 ± 5</td>
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<td>97 ± 21</td>
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<td>81 ± 21</td>
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<td>82 ± 21</td>
<td>96 ± 22</td>
<td>100 ± 20†</td>
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<td>60 ± 21</td>
<td>64 ± 21</td>
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<td>57 ± 21</td>
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<td>68 ± 21</td>
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<td>80 ± 17</td>
<td>74 ± 16*</td>
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<td>82 ± 16</td>
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<td>55 ± 16</td>
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<td>94 ± 16*</td>
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<td>73 ± 12</td>
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<td>52 ± 9</td>
<td>62 ± 9*</td>
<td>65 ± 9*</td>
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— = Not measured at this point.
†Value differs significantly between treatment groups.
See Figure 1 for precise timing of measurements.
dog in the diphenhydramine group had a low, but detectable, plasma diphenhydramine concentration (14 ng/mL) prior to premedication (Table 2). This dog had received from the owner a diphenhydramine dose of 2.4 mg/kg (1.1 mg/lb), PO, approximately 5 hours before the first blood sample was obtained. Another 2 dogs in the control group had received diphenhydramine, PO, within the 12 hours prior to premedication. One of these dogs had received diphenhydramine at 0.73 mg/kg (0.33 mg/lb), PO, 1 hour prior to blood sample collection, and the other dog received 1.5 mg/kg (0.68 mg/lb), PO, 4 hours prior to blood collection. However, diphenhydramine plasma concentrations were below the limit of quantification at all times in all dogs in the control group and remained undetectable.

No significant differences in plasma histamine concentration were identified among measurement points between or within the diphenhydramine and control groups (Table 3). Although not significant, higher histamine concentrations than at other points were measured at H3 (mean ± SD time after treatment, 24 ± 10 minutes) and H4 (80 minutes ± 16 minutes) in the diphenhydramine group and at H4 (86 ± 24 minutes) in the control group.

Table 2—Mean ± SD plasma histamine concentrations at various points for dogs anesthetized for MCT excision that received diphenhydramine (1 mg/kg, IV; n = 8).

<table>
<thead>
<tr>
<th>Measurement point*</th>
<th>No. of dogs†</th>
<th>Concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1</td>
<td>1</td>
<td>14 ± 0</td>
</tr>
<tr>
<td>D2</td>
<td>8</td>
<td>343 ± 152</td>
</tr>
<tr>
<td>D3</td>
<td>8</td>
<td>128 ± 62</td>
</tr>
<tr>
<td>D4</td>
<td>8</td>
<td>74 ± 37</td>
</tr>
<tr>
<td>D5</td>
<td>8</td>
<td>53 ± 40</td>
</tr>
<tr>
<td>D6</td>
<td>6</td>
<td>27 ± 26</td>
</tr>
</tbody>
</table>

*Measurement points included before premedication (D1); 5 minutes after treatment administration, at T1 (D2); 30 minutes after treatment administration, between T3 and T4 (D3); during peak dissection of the tumor, at T6 (D4); just before complete tumor excision, at T7 (D5); and 2 hours after extubation, at T9 (D6). †When the number of dogs is < 8, this indicates that the concentration was not detected or the value was lower than limit of quantification of the assay (10 ng/mL).
Table 3—Plasma histamine concentrations (ng/mL) at various points for the dogs in Table 1.

<table>
<thead>
<tr>
<th>Measurement pointa</th>
<th>Treatment group</th>
<th>No. of dogs</th>
<th>Mean ± SD</th>
<th>Median</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>Diphenhydramine</td>
<td>6</td>
<td>1.49 ± 1.71</td>
<td>0.74</td>
<td>0.23–4.15</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>0.91 ± 0.84</td>
<td>0.66</td>
<td>0.18–2.09</td>
<td></td>
</tr>
<tr>
<td>H2</td>
<td>Diphenhydramine</td>
<td>8</td>
<td>0.73 ± 0.78</td>
<td>0.50</td>
<td>0.11–1.61</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>0.39 ± 0.26</td>
<td>0.39</td>
<td>0.20–0.65</td>
<td></td>
</tr>
<tr>
<td>H3</td>
<td>Diphenhydramine</td>
<td>7</td>
<td>6.60 ± 11.54</td>
<td>1.13</td>
<td>0.68–20.77</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>0.87 ± 0.93</td>
<td>0.61</td>
<td>0.08–2.29</td>
<td></td>
</tr>
<tr>
<td>H4</td>
<td>Diphenhydramine</td>
<td>6</td>
<td>4.56 ± 7.81</td>
<td>1.40</td>
<td>0.22–17.07</td>
</tr>
<tr>
<td>Control</td>
<td>8</td>
<td>2.48 ± 4.01</td>
<td>0.67</td>
<td>0.19–5.04</td>
<td></td>
</tr>
<tr>
<td>H5</td>
<td>Diphenhydramine</td>
<td>6</td>
<td>0.45 ± 0.48</td>
<td>0.20</td>
<td>0.09–1.15</td>
</tr>
<tr>
<td>Control</td>
<td>7</td>
<td>0.45 ± 0.48</td>
<td>0.29</td>
<td>0.16–0.76</td>
<td></td>
</tr>
</tbody>
</table>

aMeasurement points included before premedication (H1); after anesthetic induction, at T0 (H2); at maximum manipulation of the tumor during surgical preparation, at T3 (H3); just before complete tumor excision, at T7 (H4); and 2 hours after extubation, at T9 (H5). bWhen the number of dogs is < 8, this indicates that the concentration was not detected or the value was lower than limit of quantification of the assay (0.055 ng/mL).

CI = Confidence interval.

See Figure 1 for definitions and precise timing of other measurements.

Discussion

The present study was conducted to evaluate cardiorespiratory responses during isoflurane anesthesia in dogs undergoing MCT excision with or without IV diphenhydramine administration, with the intent of identifying the occurrence of hypotension and other adverse events. Our hypothesis was that diphenhydramine administration would minimize significant variations in blood pressure associated with the changes in plasma histamine concentration during surgical manipulation of MCTs; however, no clear benefit of diphenhydramine administration was observed, given that plasma histamine concentrations and possible related adverse effects were similar in both treatment groups.

The dose of diphenhydramine used in this study (1 mg/kg, IV) was effective in achieving plasma concentrations for a duration that should have counteracted the effects of histamine throughout surgery. A mean ± SD maximum plasma diphenhydramine concentration of 343 ± 152 ng/mL was achieved 5 minutes after IV diphenhydramine administration. These data are similar to the peak plasma concentrations of 306 ng/mL obtained in another study involving conscious research hounds and the same dose and route of administration. In human dose-response research, plasma diphenhydramine concentrations of at least 25 ng/mL are considered therapeutic for reducing the diameter of skin wheals caused by SC histamine injection; however, similar research has not been reported for dogs.

At the time of tumor excision (83 ± 15 minutes after treatment administration), plasma concentrations were still > 25 ng/mL in 6 of the 8 dogs that received diphenhydramine in the present study. Plasma histamine concentrations necessary to cause cardiovascular responses are larger than the concentrations required to elicit cutaneous responses; therefore, it is possible that plasma diphenhydramine concentrations considered therapeutic for producing cutaneous signs are ineffective at preventing cardiovascular responses.

Diphenhydramine administration may not be effective at preventing the cardiovascular effects of histamine release in dogs undergoing MCT excision for several reasons. First, diphenhydramine at commonly recommended doses may not counteract the cardiovascular effects of histamine. In fact, research has shown that diphenhydramine alone (6 to 10 mg/kg [2.7 to 4.5 mg/lb]) abolishes the vascular depressor effects of low doses of exogenous histamine in anesthetized dogs and cats, but not the severe cardiovascular depression caused by higher histamine doses. Second, plasma histamine concentrations in dogs with MCT are greater than those in healthy dogs but not necessarily associated with adverse cardiovascular effects. Plasma histamine concentrations in healthy dogs (< 0.8 ng/mL) are typically lower than in dogs with MCTs (mean ± SD, 2.9 ± 2.2 ng/mL); however, no differences in plasma histamine concentrations were identified between healthy dogs (median, 0.19 ng/mL; range, 0.12 to 0.36 ng/mL) and dogs with MCTs (median, 0.39 ng/mL; range, 0.11 to 2.75 ng/mL) in 1 study, although dogs with gross disease in that study had significantly higher plasma histamine concentrations (median, 0.73 ng/mL; range, 0.26 to 2.75 ng/mL) than did healthy dogs.

Plasma histamine concentrations in both treatment groups in the present study were similar throughout anesthesia, with mean values ranging between 0.45 and 6.60 ng/mL in the diphenhydramine group and 0.39 and 2.48 ng/mL in the control group. The higher concentrations were typically present during surgical manipulation of the tumor (T6 to T8); however, they were generally lower than those associated with hypotension in other studies. In one of those studies, dogs anesthetized with sodium pentobarbital that received morphine (1 mg/kg, IV) had plasma histamine concentrations that increased to 100 to 300 ng/mL and were associated with a decrease from baseline in MAP of 40 to 60 mm Hg in all
dogs. Morphine is known for its histamine-releasing properties in dogs as it causes mast cell degranulation. In another study, phenobarbital-anesthetized dogs that received exogenous histamine had subsequent plasma histamine concentrations of 17.4 to 21.4 ng/mL, which were associated with a significant decrease in MAP (median decreased to 63 mm Hg from 120 mm Hg) and total systemic vascular resistance (median decreased to 0.55 mm Hg/mL/kg/min [0.25 mm Hg/mL/lb/min] from 1.09 mm Hg/kg/min [0.50 mm Hg/mL/lb/min]). The differences between the histamine concentrations in these studies and those measured in the present study likely account for the lack of clinically important hypotension observed in our study.

A third reason for the lack of specificity and ineffectiveness of diphenhydramine at preventing the vascular actions of histamine in the present study could have been that vasodilation from histamine is mediated by both H₁ and H₂ receptors in pulmonary, cerebral, and systemic vascular beds. For this reason, simultaneous administration of both H₁ and H₂ receptor antihistamines has been recommended to prevent vasodilation from histamine. Inactivation of the histamine-3 receptor also appears to contribute to cardiovascular dysfunction in anaphylaxis. In addition, prostaglandin D₂ plays a role in mediating hypotension in humans with mast cell diseases and this mediator is also released by canine mast cells, with actions that are not prevented by antihistamine treatment. Because of diphenhydramine-specific actions on H₁ receptors, its protection against histamine effects may be limited in dogs with MCTs.

In the present study, dogs in the control group had significantly greater MAP and DAP values during surgical manipulation of the tumor (T6 to T8). These results contradict the supposition that diphenhydramine administration will help prevent the adverse effects of histamine and improve cardiovascular function in dogs with MCTs undergoing surgery. Despite similar values for SVI and cardiac index in both treatment groups, except for a higher cardiac index value at T7 in the diphenhydramine group, the increases in HR, MAP, and DAP in the control group in association with surgical stimulation were not observed in the diphenhydramine group. Interestingly, the 1 dog in the control group that had an MAP < 60 mm Hg for > 10 minutes required dopamine administration for only 7 minutes, given that MAP improved as soon as surgery started. On the other hand, the 1 hypotensive dog in the diphenhydramine group required dopamine for the duration of anesthesia despite surgical stimulation. Hence, these findings, despite small numbers, provided no support for a protective effect of diphenhydramine against development of hypotension during MCT excision in dogs.

Dogs that received diphenhydramine in the present study could have had fewer sympathetic nervous responses to surgical stimulation than dogs in the control group owing to inverse agonism of H₁ receptors and the consequent decrease in sympathetic nerve transmission. Histamine is released pre- and post-synaptically in terminals of peripheral sympathetic nerves, where it may act as a primary neurotransmitter or modulate norepinephrine release in dogs, mice, and guinea pigs. Findings in mice and guinea pigs indicate that H₁ receptors play a major role in the regulation of sympathetic nerve activity and that histamine potentiates synaptic transmission in sympathetic ganglia through H₁ activation. Baroreflex HR response is attenuated in H₁ receptor-null mice and in mice treated with H₁-receptor antihistamines, indicating that sympathetic nerve activity is reduced when H₁ receptors are blocked or absent.

The transient hypotension that occurred during anesthesia in some of the dogs in the present study may also have been the result of dose-dependent vasodilatory effects of isoflurane. Isoflurane end-tidal concentrations of 1.5% exceed the 1.0 MAC value for isoflurane (1.28%), and in combination with the premedication and induction anesthetics, may exacerbate cardiovascular depression from synergistic effects and MAC-sparing effects. Hydromorphone (0.1 mg/kg, IV) reduces the MAC of isoflurane in dogs by 50% at 1.5 hours and by 33% at 4.5 hours after administration. To minimize effects of the anesthetic protocol on the study results, we avoided drugs associated with histamine release. Both hydromorphone and propofol have been associated with no or minimal histamine release in healthy dogs in various studies.

In contrast to the contribution of isoflurane to hypotension, both isoflurane and halothane can interfere and prevent the vasodilative effects of histamine. Histamine increases the intracellular concentration of calcium ions in endothelial cells, which leads to activation of endothelial nitric oxide synthase and the production and actions of nitric oxide, which is a potent vasodilator. Isoflurane and halothane decrease the influx of calcium ions and prevent nitric oxide production and histamine-mediated vasodilation. Therefore, isoflurane may actually protect against histamine-related actions in dogs with MCT.

Eight dogs were used in each treatment group in the present study, which may have been too few to avoid type II error in comparisons between the 2 groups in this clinical scenario. However, an a priori sample size calculation had been performed and was based on a difference between groups of 15 mm Hg in MAP (SD, 10 mm Hg; α = 0.05; power = 0.90), which indicated a required sample size of 8 dogs/group. Anesthetic protocols were identical in both groups in the present study to provide a similar anesthetic depth and limit the influence of the protocols on cardiorespiratory function. It could be argued that dogs in the diphenhydramine group may have been influenced by the effects of diphenhydramine on the CNS, given that this drug can cross the blood-brain barrier and cause drowsiness in humans. However, diphenhydramine caused no sedative effects in dogs in 2 studies. In 1 study, doses of 1 mg/kg, IV, and 2 mg/kg, IM were administered. The other study
was a randomized clinical trial involving doses of 2, 4, or 8 mg/kg, IM, administered prior to anesthesia. These findings make the effect of diphenhydramine on inhalant requirements in the present study less likely.

Dogs with MCTs that received diphenhydramine IV as a preemptive treatment prior to tumor excision in the present study had cardiorespiratory responses similar to dogs that received saline solution, and some variables (MAP and DAP) were better in the control group at the time of peak dissection of the tumor, despite a lower cardiac index value. Despite these differences, cardiorespiratory values in both groups were considered within acceptable limits for anesthetized dogs. In addition, no clear benefit of diphenhydramine administration was evident for dogs with MCTs given that plasma histamine concentrations and possible related adverse effects were similar in both groups during MCT manipulation and excision in isoflurane-anesthetized dogs.

Acknowledgments

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Footnotes

a. Sandoz Canada Inc, Boucherville, QC, Canada.
b. BD Insyte-W, BD Infusion Therapy Systems Inc, Sandy, Utah.
c. pms-Propofol (1%), PharmaScience, Montreal, QC, Canada.
d. Aerrane Isoflurane USP, Baxter Corp, Mississauga, ON, Canada.
e. EMC 2002E1, Hallowell, Pittsfield, Mass.
f. Transducer set, Becton Dickinson Critical Care System Pte Ltd, Singapore.
g. CareScape B650 monitor, GE Healthcare, Helsinki, Finland.
h. Novametrix Medical Systems, Wallingford, Conn.
i. ABL 800Flex series analyzer, Radiometer, Copenhagen, Denmark.
k. Diphenhydramine hydrochloride injection USP (50 mg/mL), Pharmaceutical Partners of Canada Inc, Richmond Hill, ON, Canada.
l. Teva Canada Ltd, Toronto, ON, Canada.
m. Waters Alliance 2695 HPLC separation system and 2475 multiwavelength fluorescence detector, Waters, Mississauga, ON, Canada.
n. Empower 2, Waters, Mississauga, ON, Canada.
o. Histamine enzyme immunoassay IM2015, Immunootech, Marseille, France.
p. PROC MIXED, SAS, version 9.3, SAS Institute, Cary, NC.
q. PROC NPARiWAY, SAS, version 9.3, SAS Institute, Cary, NC.

References


