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Impact of cytidine diphosphocholine on oxygenation in client-owned dogs with aspiration pneumonia

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Abstract

Background: New drugs for veterinary patients with acute respiratory distress syndrome (ARDS) are urgently needed. Early or late postinfection treatment of influenza-infected mice with the liponucleotide cytidine diphosphocholine (CDP-choline) resulted in decreased hypoxemia, pulmonary edema, lung dysfunction, and inflammation without altering viral replication. These findings suggested CDP-choline could have benefit as adjunctive treatment for ARDS in veterinary patients (VetARDS).

Objectives: Determine if parenterally administered CDP-choline can attenuate mild VetARDS in dogs with aspiration pneumonia.

Animals: Dogs admitted to a veterinary intensive care unit (ICU) for aspiration pneumonia.

Methods: Subjects were enrolled in a randomized, double-blinded, placebo-controlled trial of treatment with vehicle (0.1 mL/kg sterile 0.9% saline, IV; n = 8) or CDP-choline (5 mg/kg in 0.1 mL/kg 0.9% saline, IV; n = 9) q12h over the first 48 hours after ICU admission.

Results: No significant differences in signalment or clinical findings were found between placebo- and CDP-choline-treated dogs on admission. All dogs exhibited tachycardia, tachypnea, hypertension, hypoxemia, hypocapnia, lymphopenia, and neutrophilia. CDP-choline administration resulted in rapid, progressive, and clinically relevant increases in oxygenation as determined by pulse oximetry and ratios of arterial oxygen partial pressure (P_aO_2 mmHg) to fractional inspired oxygen (% F_iO_2) and decreases in alveolar-arterial (A-a) gradients that did not occur in placebo (saline)-treated animals. Treatment with CDP-choline was also associated with less platelet consumption over the first 48 hours, but had no detectable detrimental effects.

Conclusions and Clinical Importance: Ctyidine diphosphcholine acts rapidly to promote gas exchange in dogs with naturally occurring aspiration pneumonia and is a potential adjunctive treatment in VetARDS patients.

Abbreviations: A-a gradient, alveolar-arterial oxygen gradient; ARDS, acute respiratory distress syndrome; ATII cell, alveolar type II respiratory epithelial cell; CDP-choline, cytidine diphosphocholine; F_iO_2, fraction of O_2 in inspired gas (0.21 in room air); IAV, influenza A virus; ICU, intensive care unit; LPN, liponucleotide; P:F ratio, P_aO_2:F_iO_2 ratio; P_aO_2, arterial partial pressure of O_2; S_pO_2, peripheral arterial O_2 saturation.

KEYWORDS
alveolar type II cell, ARDS, influenza, P:F ratio, phospholipid, surfactant

1 | INTRODUCTION

The acute respiratory distress syndrome (ARDS), which is characterized by acute severe hypoxemia, decreased lung compliance, and the presence of non-cardiogenic pulmonary edema is a relatively common sequela of lung injury and remains a clinically important problem in both human and veterinary critical care medicine.[1] Diagnostic criteria for ARDS in dogs (VetARDS) were developed in 2007 by the Dorothy Havenmeyer Working Group and are similar to those in the original American-European Consensus Conference (AECC) definition of human ARDS.[2] However, mortality rates in veterinary patients are far higher (84%-100%) than in humans (35%-40%), primarily because the cost of care for mechanical ventilation in dogs is substantial (approximately $15 000) and owners often elect euthanasia instead of treatment.[3] Indeed, even in dogs that are mechanically ventilated survival rates are very low.[4,5] Hence, new drugs that will decrease morbidity and mortality in dogs with VetARDS are urgently needed.

Development of severe ARDS in mice infected with H1N1 influenza A virus (IAV) resulted in alveolar type II (ATII) epithelial cell dysfunction and altered phospholipid metabolism.[6-9] Moreover, the liponucleotide (LPN) cytidine 5′-diphosphocholine (CDP-choline), which is an essential precursor for de novo phosphatidylcholine synthesis,[10,11] was undetectable in ATII cells from IAV-infected mice.[9] Importantly, early or late postinfection treatment of mice with cytidine diphosphocholine (CDP)-choline decreased IAV-induced hypoxemia, pulmonary edema, lung dysfunction, and inflammation without altering viral replication.[12] Because LPNs are inexpensive and known to be very safe in both dogs and humans,[13,14] these findings were very promising.[15]

We hypothesized that early post-admission CDP-choline treatment would improve oxygenation in dogs with VetARDS and might therefore be a useful adjunct treatment in this population. To test this hypothesis, we conducted a randomized double-blinded, placebo-controlled clinical trial of IV CDP-choline in client-owned pet dogs admitted to the small animal intensive care unit (ICU) at The Ohio State University Veterinary Medical Center (OSUVMC) for aspiration pneumonia. Our objectives were to determine the impact of treatment on gas exchange and to show that CDP-choline did not have detrimental effects in this population.

2 | METHODS

2.1 | Approvals

All animal experiments complied with the National Research Council *Guide for the Care and Use of Laboratory Animals* and were approved by the Institutional Animal Care and Use Committee and OSUVMC Clinical Trials Office.

2.2 | Study enrollment criteria

We enrolled client-owned dogs of both sexes and any breed that had all of the following: (1) An identifiable proximate cause for aspiration pneumonia; (2) A consistent diagnosis based on clinical signs (acute onset dyspnea or tachypnea at rest, persistent panting not related to hyperthermia, exercise intolerance, coughing, anorexia, malaise, or other signs of systemic illness) and radiographic changes (cranioventral distribution of parenchymal infiltrates exhibiting interstitial or alveolar patterns, most commonly affecting the right middle lung lobe); (3) Dyspnea or tachypnea that improved with the initiation of supplemental O_2; and (4) Evidence of moderate to severe hypoxemia or pulmonary dysfunction characterized by low arterial dissolved oxygen levels (P_aO_2 <70 mmHg), increased alveolar-arterial gradients (A-a gradient >30 mmHg), low ratios of arterial dissolved oxygen to fractional inspired oxygen (P_aO_2:F_iO_2 ratio <350), or some combination of these obtained via arterial blood gases obtained on room air. Additional enrollment criteria were that enrolled dogs should be between 6 months and 12 years old, weigh >5 kg but not be considered severely underweight or obese for the breed (ie, body condition score ≤2 or ≥8, respectively),[16] and not be aggressive or intractable when handled.

2.3 | Study exclusion criteria

Dogs were excluded from the study, for failure to meet enrollment criteria or presence of clinically relevant chronic co-morbidities (eg, neoplasia, cardiovascular disease, advanced neurological disease).

2.4 | Informed consent

Written consent was obtained from all owners, who received a $400 financial incentive (contribution toward hospital expenses) to participate.

2.5 | Treatment groups

The number of dogs enrolled was based on availability rather than a priori analysis of effect size. Dogs were randomized to receive an IV bolus of 0.1 mL/kg sterile 0.9% saline (placebo group) or an IV bolus of 0.1 mL/kg saline containing 5 mg/kg CDP-choline q12h. Treatment

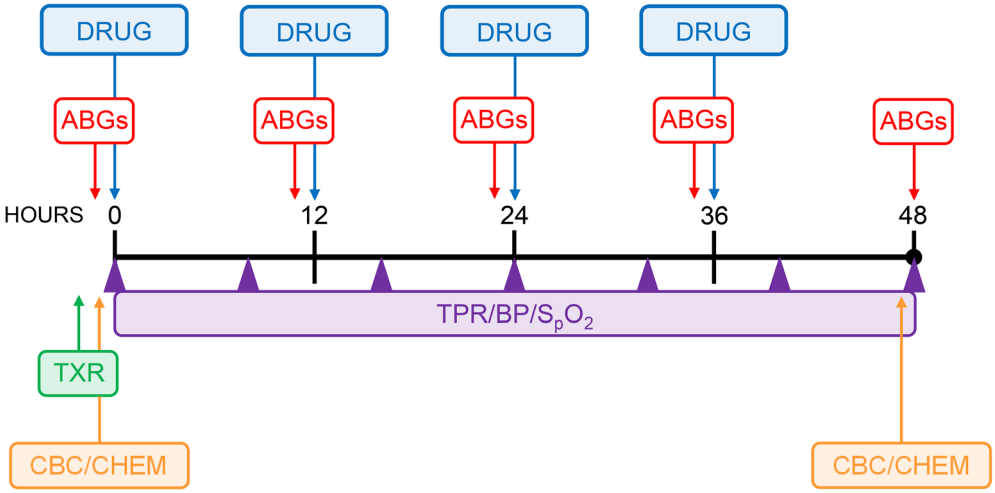

FIGURE 1 Liponucleotide clinical trial protocol. After randomization to placebo or cytidine diphosphocholine-choline treatment groups, demographic data, vital signs (temperature [T], pulse rate [P], respiratory rate [R]), and blood pressure (BP) were recorded. A thoracic radiograph (TXR) was taken. Peripheral arterial O_2 saturation (S_pO_2) was measured by pulse oximetry at a non-pigmented, hair free skin surface or mucous membrane (most often the inner lip). Initial arterial and venous blood samples were collected (from the dorsal pedal or femoral artery and the cephalic or saphenous vein, respectively). Collected blood samples were used to obtain arterial blood gases (ABGs), complete blood count (CBC), and clinical chemistry (CHEM) data. Each dog was then given its first dose of the relevant test agent (DRUG), which was provided in de-identified vials. Subsequently, vital signs (TPR), BP, and S_pO_2 were recorded every 8 hours. Arterial blood was collected every 12 hours to measure ABGs immediately prior to administration of the next dose of the test agent. At 48 hours, a second venous blood sample was collected for CBC and CHEM

vials were prepared and de-identified by a member of the research team that was not involved in patient management.

2.6 | Monitoring

Vital signs (body temperature, pulse rate, and respiratory rate) and blood pressure were recorded q8h. The S_pO_2 was measured q8h by pulse oximetry. Arterial blood gases were measured on enrollment then q12h, immediately before drug administration. Venous blood was collected at time of enrollment and at 48 hours for CBC and serum biochemistry. A schematic of the clinical protocol is shown in Figure 1.

2.7 | Patient management

Apart from administration of test agents and increased frequency of arterial blood collection (q12h instead of q24h), all patients were managed according to best standard of care practices for the management of aspiration pneumonia. When feasible, supplemental O_2 was administered to dogs <40 kg in a Snyder ICU oxygen cage (Snyder Mfg. Co., Centennial, Colorado), which allows for environmental control (ie, internal temperature and humidity) and controlled O2 concentrations up to an F_iO_2 of 0.6. For patients too large to fit comfortably into the Snyder ICU cage, a single or double nasal cannula was placed at the level of the oropharynx for O_2 administration at approximately 100 to 150 mL/kg/min. Oxygen supplementation using this approach introduced more variability depending on the size of the patient and whether the animal was panting or not. For this reason, all arterial blood gas samples were obtained on room air.

Conservative IV fluid therapy was provided to prevent dehydration, most commonly using a balanced or hypotonic crystalloid (ie, Plasmalyte, LRS, 0.45% NaCl). Broad-spectrum antibiotics were often employed: the choice of single or double agent treatment was determined by the clinician, based on severity of illness, antibiotic history, existing co-morbidities, culture and susceptibility results, OSUVMC Antimicrobial Use Guidelines or some combination of these. Common antibiotics used included potentiated ampicillin/amoxicillin (Unasyn, Clavamox), fluoroquinolones (Baytril), lincosamides (Clindamycin), and doxycycline. Additional treatments were dictated by specific patient needs and included antiemetics (ondansetron, cerenia), sedatives (butorphanol, acepromazine), bronchodilators (SC terbutaline), neuromuscular agents (pyridostigmine), gastroprotectants (pantoprazole, sucralfate), and hyperosmotic inhalants (3% hypertonic saline nebulization). One dog in the drug treatment group received corticosteroids to manage airway swelling.

2.8 | Measurement of peripheral oxygen saturation (S_pO_2)

The S_pO_2 typically was measured from a non-pigmented portion of the inner lip or a non-haired portion of skin (eg, inner pinna, toe webbing, base of tail) using a Masimo Pulse Oximeter (Masimo Corp., Irvine, California).

2.9 | Measurement of arterial blood gases

Arterial blood was collected from the dorsal pedal or medial femoral artery using standard techniques on room air in dogs placed in sternal recumbency. Sedated dogs were repositioned from lateral recumbency and allowed to equilibrate before arterial blood collection. Blood gases were measured using a Stat Profile Prime Plus VET Critical Care Analyzer (Nova Biomedical, Waltham, Massachusetts) according to the manufacturer's instructions.

2.10 | Measurement of blood pressure

Blood pressure measurement in dogs was obtained from the forelimb while in sternal recumbency using oscillometric methods. Technique and cuff size selection was chosen on an individual basis according to the American College of Veterinary Internal Medicine consensus statement: Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats.[17]

2.11 | Clinical chemistry

Blood samples were analyzed in the Clinical Chemistry Laboratory at the OSUVMC. Serum biochemical profiles were performed using a Cobas c501 Automatic Chemistry Analyzer (Roche Diagnostics, Indianapolis, Indiana). Plasma electrolyte concentrations were measured using a Nova phOX Analyzer (Nova Biomedical). Plasma osmolality was determined using a Multi-osmette osmometer (Fisher Scientific, Waltham, Massachusetts). All assays were performed according to the manufacturers' instructions using standardized protocols validated for veterinary use. Quarterly quality assurance testing is performed as part of a program administered by the Veterinary Laboratory Association. Commercial controls and calibrators are evaluated on a regular basis.

2.12 | Hematology

Blood samples were analyzed in the Hematology Laboratory at the OSUVMC. Complete blood counts were determined using an Advia 2120i Hematology Analyzer (Siemens Healthineers, Plymouth, Massachusetts). For differential cell counts, blood films were stained with Wright-Giemsa using a Harleco Midas II Automated Stainer (Millipore Sigma, St. Louis, Missouri). All assays were performed according to manufacturer's instructions using standardized protocols validated for veterinary use. Quarterly quality assurance testing, which includes peer review of blood films, is performed as part of a program administered by the Veterinary Laboratory Association. Commercial controls and calibrators are regularly evaluated.

2.13 | Criteria for termination of the trial

Termination criteria were evidence of harm in CDP-choline-treated dogs or clear evidence of futility. Evidence of harm was any adverse event that could be attributed to study drug administration such as local or systemic anaphylaxis, injection site irritation, acute changes in vital parameters that were not attributed to underlying disease, or acute alterations in laboratory findings that could not be explained by underlying illness. Adverse events were tracked using the Veterinary Cooperative Oncology Group-Common Terminology Criteria for Adverse Events (VCOG-CTCAE) after investigational treatment in dogs and cats.[18] Criteria for trial termination were not reached in any enrolled subject.

2.14 | Clinical data management

Clinical trial data were collected and managed using Research Electronic Data Capture (REDCap) electronic data capture tools hosted at The Ohio State University. Five-variable Acute Patient Physiologic and Laboratory Evaluation (APPLE) scores[19] were calculated for each patient based on data collected at the time of admission. These scores are based on plasma glucose, albumin, and lactate concentrations, platelet count, and mentation score.

2.15 | Statistical analyses

Descriptive statistics were calculated using Instat software (GraphPad, San Diego, California). Gaussian data distribution was verified by the method of Kolmogorov and Smirnov. All data are shown as mean ± SD (SD) or as box and whisker plots, in which data in the box represent first quartile, median, and third quartile for each treatment group and whiskers indicate highest and lowest sample values within the group. Statistical analyses of datasets were performed using paired or unpaired Student's *t*-tests (for 2 groups) or analysis of variance (ANOVA) with a post hoc Tukey-Kramer multiple comparison post-test (for ≥3 groups). Between group (drug vs. placebo) tests were unpaired whereas within group (drug or placebo) tests were paired for each subject. $P < .05$ was considered significant.

3 | RESULTS

3.1 | Characteristics of the study population

Over the course of 2 years, we enrolled 18 dogs with aspiration pneumonia into a 48-hour randomized, double-blinded placebo-controlled clinical trial of CDP-choline (Figure 1). Aspiration pneumonia was most commonly a secondary complication of vomiting or regurgitation. Underlying comorbidities that definitively or presumably contributed to the development of aspiration pneumonia included acute

TABLE 1 Demographics of intensive care unit-admitted dogs, randomized to receive IV saline placebo or IV CPD-choline for the treatment of aspiration pneumonia.

	Placebo[a]	Drug[b]
Age (years)[c]	8.8 ± 3.9	4.9 ± 4.4
Number of breeds	6[d]	10[e]
Body weight (kg)[c]	28.6 ± 19.2	28.2 ± 15.5
Intact male dogs	0	2
Neutered male dogs	6	3
Intact female dogs	0	2
Neutered female dogs	2	3

[a]0.1 mL/kg sterile 0.9% saline, IV, q12h, n = 8.
[b]CDP-choline (5 mg/kg) in 0.1 mL/kg sterile 0.9% saline, IV, q12h, n = 10.
[c]Data shown as mean ± SD.
[d]English Bulldog, Great Dane, Labrador Retriever (3), Mixed, Standard Poodle, Welsh Corgi.
[e]Bernese Mountain Dog, Dachshund, English Bulldog, German Shepherd, Mastiff, Mixed, Newfoundland, Rhodesian Ridgeback, Springer Spaniel, Whippet.

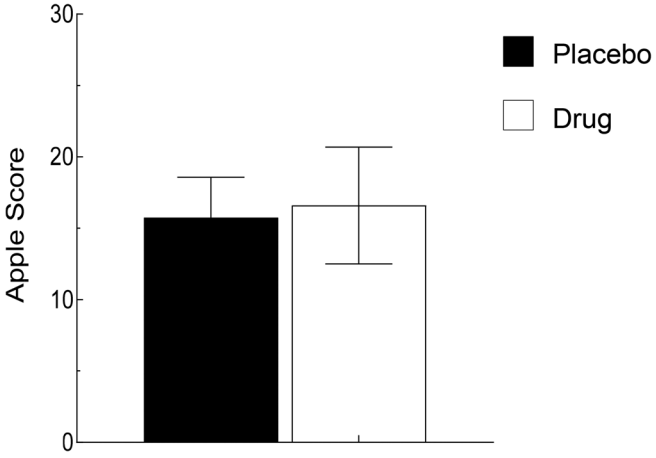

FIGURE 2 Severity of illness did not differ between groups at the time of intensive care unit admission. Acute Patient Physiologic and Laboratory Evaluation[19] scores at the time of admission for dogs assigned to placebo and drug treatment groups. Data are shown as mean ± SD (SD). Data were analyzed by unpaired Student's *t*-test

self-limiting vomiting (2 dogs), idiopathic megaesophagus (1 dog), brachycephalic airway syndrome (2 dogs), esophageal dysmotility (3 dogs), and degenerative polyneuropathy (1 dog). Dogs in both groups were treated with an average of 2 antibiotics and no significant difference in antibiotic usage was found between groups. Likewise, no significant difference was found in number of additional treatments administered between dogs in the placebo group (7 ± 2) and those in the drug treatment group (9 ± 1).

Dogs were randomized to receive placebo (n = 8) or CDP-choline treatment (n = 10): clinicians were blinded to randomization. No significant differences in age, breed, or sex distribution were found between groups (Table 1). Likewise, no significant differences were found in 5-variable APPLE scores at the time of admission (Figure 2). Dogs in both groups were maintained on supplemental O_2 for similar time periods. Two dogs in each group ultimately were euthanized because of deteriorating clinical condition, but not in the first 48 hours after enrollment.

3.2 | Intravenous CDP-choline improved cardiopulmonary function in dogs with aspiration pneumonia

At the time of admission, all 18 dogs exhibited modest tachycardia, tachypnea, and hypertension (Table 2). Dogs in both treatment arms were comparably desaturated (mean S_pO_2 < 92% on room air). At 48 hours, dogs in both treatment arms were no longer tachycardic or significantly tachypneic but remained hypertensive. Importantly, mean S_pO_2 was significantly higher in CDP-choline-treated dogs than in placebo controls at this timepoint and at the low end of the normal range (>95%).

To further parse these data, we performed paired analysis of data for each individual dog at 0, 24, and 48 hours after enrollment. Unlike administration of placebo, which had no beneficial effects on S_pO_2, CDP-choline treatment resulted in improved S_pO_2 at 48 hours (Figure 3A). This effect remained significant even when the dog that presented with severe hypoxemia was excluded. Heart rates were significantly lower in drug-treated dogs at 24 hours, but did not decrease in placebo-treated controls until 48 hours (Figure 3B).

3.3 | Intravenous CDP-choline improved gas exchange in dogs with aspiration pneumonia

The P:F and S_pO_2:F_iO_2 ratios are closely correlated and P:F ratios can be imputed from S_pO_2 values.[20,21] However, S_pO_2 data provide no information about other parameters of gas exchange, such as A-a gradients. Consequently, we included arterial blood gas measurements q12h in our protocol, but because of poor compliance in some dogs, complete arterial blood gas datasets were only available for 6 placebo-treated and 7 CDP-choline-treated subjects. At the time of admission, dogs in both treatment arms of this subgroup were comparably hypoxemic and mildly hypocapnic (Table 3), which was consistent with their increased respiratory rates (Table 2). Mean A-a gradients were markedly increased (and to a similar extent) in both groups on admission, which is suggestive of either ventilation-perfusion mismatch or intrapulmonary shunting.[22] However, dogs were not acidotic and had normal anion gaps.

The P_aCO_2, pH_a, and anion gap results did not change significantly over the first 48 hours in either treatment group (Table 3). However, administration of CDP-choline resulted in a progressive increase in P:F ratios (calculated on room air), which was significant by 24 hours (Figure 4A). The mean P:F ratio in the drug-treated group increased from 300 mmHg at the time of enrollment to 365 mmHg at 24 hours and 372 mmHg at 48 hours. Likewise, although they did not return to normal (<15 mmHg), A-a gradients significantly decreased in CDP-choline-treated dogs over the first 48 hours (Table 3 and

TABLE 2 Vital signs upon admission and at 48 hours in intensive care unit-admitted dogs, randomized to receive IV saline placebo or IV CDP-choline for the treatment of aspiration pneumonia.

	Placebo[a]		Drug[b]		
	Admission	48 hours	Admission	48 hours	Reference
Body temperature (°F)	102.5 ± 2.0	101.3 ± 1.7	102.4 ± 1.3	101.5 ± 1.9	99.5-102.5
Heart rate (beats/min)	**126 ± 25**	86 ± 14[§]	**130 ± 22**	94 ± 19[‖]	60-120
Respiratory rate (breaths/min)	**41 ± 11**	38 ± 17	**50 ± 19**	**41 ± 19**	12-40
Mean arterial pressure (mmHg)	**136 ± 28**	**145 ± 25**	**141 ± 32**	**147 ± 25**	80-120
Peripheral O_2 saturation (%)	**90.9 ± 3.1**	**91.3 ± 10.5**	**90.8 ± 6.3**	95.4 ± 3.2[‡]	95-98

Note: Data shown as mean ± SD. Bold font indicates mean value outside reference range.
[‡]$P < .05$, [§]$P < .005$, [‖]$P < .001$, versus time of admission for that group, by paired Student's *t*-test.
[a]0.1 mL/kg sterile 0.9% saline, IV, q12h, n = 8.
[b]CDP-choline (5 mg/kg) in 0.1 mL/kg sterile 0.9% saline/kg, IV, q12h, n = 10.

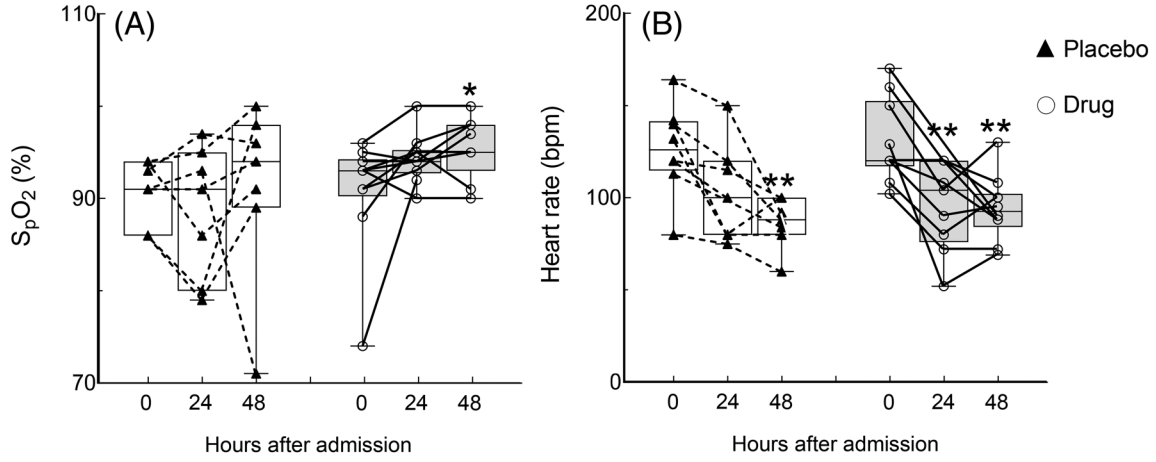

FIGURE 3 Intravenous cytidine diphosphocholine (CDP)-choline improves cardiopulmonary function in dogs with aspiration pneumonia. Effect of treating dogs with aspiration pneumonia with placebo (0.1 mL/kg sterile 0.9% saline, i.v.) or drug (CDP-choline, 5 mg/kg in 0.1 mL/kg sterile 0.9% saline, i.v.) every 12 hours over the first 48 hours after admission to the OSUVMC Small Animal intensive care unit on: (A) Peripheral arterial O_2 saturation (S_pO_2, %); and (B) Heart rate (beats per minute [bpm]). n = 8 placebo-treated dogs and 10 drug-treated dogs. S_pO_2 values were collected in room air without supplemental oxygenation ($F_iO_2 = 0.21$). Data in box represent first quartile, median, and third quartile for each treatment group. Whiskers indicate highest and lowest sample values within the group. Data for each individual dog are linked over time by connecting lines. All data were analyzed by paired ANOVA with a post hoc Tukey-Kramer multiple comparison post-test. *$P < .05$, **$P < .005$, versus time 0 in same treatment group

TABLE 3 Arterial blood gas parameters upon admission and at 48 hours in intensive care unit-admitted dogs, randomized to receive IV saline placebo or IV CDP-choline for the treatment of aspiration pneumonia.

	Placebo[a]		Drug[b]		
	Admission	48 hours	Admission	48 hours	Reference
P_aO_2:F_iO_2 (mmHg)	**322 ± 42**	**343 ± 73**	**300 ± 50**	**372 ± 71**[§]	>400
P_aCO_2 (mmHg)	**23.9 ± 3.7**	**29.0 ± 4.7**[‡]	**26.8 ± 4.2**	**30.1 ± 6.6**	32-43
A-a gradient (mmHg)	**47.3 ± 11.5**	**38.8 ± 15.4**	**51.6 ± 13.5**	**31.4 ± 15.6**[‖]	<15
pH_a	7.46 ± 0.02	7.43 ± 0.02	7.43 ± 0.05	7.42 ± 0.03	7.35-7.46
Anion Gap (mEq/L)	16.3 ± 4.7	19.2 ± 4.4	19.6 ± 6.6	17.9 ± 3.2	15-25

Note: Data shown as mean ± SD. Bold font indicates mean value outside reference range.
[‡]$P < .05$, [§]$P < .005$, [‖]$P < .001$, versus time of admission for that group, by paired Student's *t*-test.
[a]0.1 mL/kg sterile 0.9% saline, IV, q12h, n = 6.
[b]CDP-choline (5 mg/kg) in 0.1 mL/kg sterile 0.9% saline/kg, IV, q12h, n = 7.

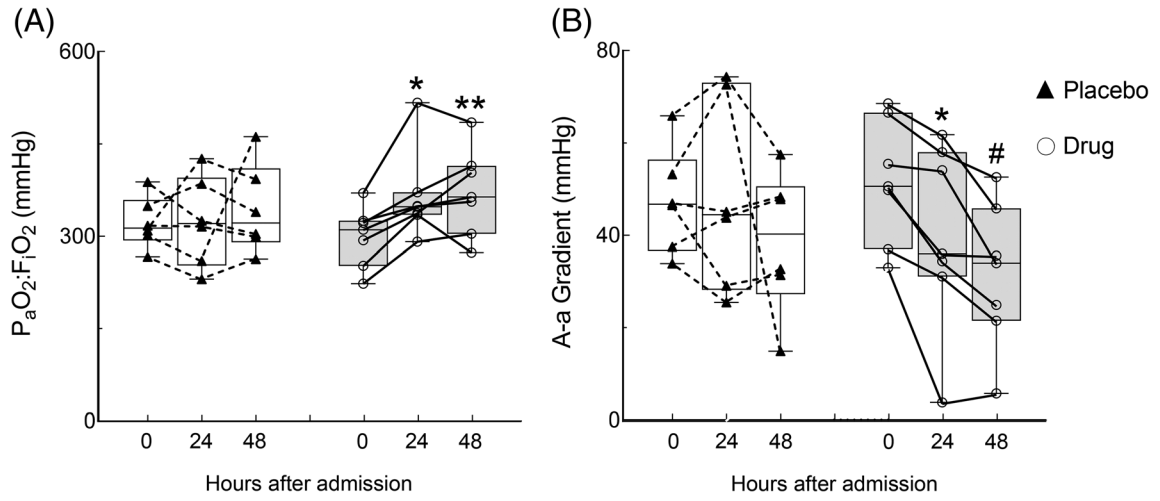

FIGURE 4 Intravenous cytidine diphosphocholine (CDP)-choline improves pulmonary gas exchange in dogs with aspiration pneumonia. Effect of treating dogs with aspiration pneumonia with placebo (0.1 mL/kg sterile 0.9% saline, i.v.) or drug (CDP-choline, 5 mg/kg in 0.1 mL/kg sterile 0.9% saline, i.v.) every 12 hours over the first 48 hours after admission to OSUVMC Small Animal intensive care unit on: (A) P_aO_2:F_iO_2 ratio (mmHg); and (B) A-a gradient (mmHg). n = 6 placebo-treated dogs and 7 drug-treated dogs. P_aO_2 values were collected in room air without supplemental oxygenation (F_iO_2 = 0.21). Data in box represent first quartile, median, and third quartile for each treatment group. Whiskers indicate highest and lowest sample values within the group. Data for each individual dog are linked over time by connecting lines. All data were analyzed by paired ANOVA with a post hoc Tukey-Kramer multiple comparison post-test. *P < .05, **P < .005, #P < .001, versus time 0 in same treatment group

TABLE 4 Peripheral blood leukocyte and erythrocyte parameters upon admission and at 48 hours in intensive care unit-admitted dogs, randomized to receive IV saline placebo or IV CDP-choline for the treatment of aspiration pneumonia.

	Placebo[a]		Drug[b]		
	Admission	48 hours	Admission	48 hours	Reference
Monocytes ($\times 10^9$/L)	0.7 ± 0.8	0.9 ± 0.6	0.5 ± 0.3	0.9 ± 0.6‡	0.1-1.1
Lymphocytes ($\times 10^9$/L)	**0.8 ± 0.6**	1.4 ± 0.8	**0.9 ± 0.6**	4.1 ± 10.1	1.0-4.6
Segmented neutrophils ($\times 10^9$/L)	**11.9 ± 10.5**	**17.0 ± 10.2**‡	8.7 ± 4.7	**12.7 ± 7.0**	2.6-10.8
Platelets ($\times 10^9$/L)	297 ± 99	210 ± 59‡	220 ± 44‖	200 ± 47	145-463
Hematocrit (%)	44.0 ± 8.2	**38.6 ± 5.9**	45.3 ± 9.8	**36.5 ± 7.3**‡	40-59
Hemoglobin (g/dL)	15.0 ± 3.1	**13.1 ± 2.3**	15.4 ± 3.5	**12.4 ± 2.5**‡	13.5-20.4
RBC ($\times 10^{12}$/L)	6.4 ± 1.4	**5.5 ± 0.8**	6.5 ± 1.6	**5.2 ± 0.9**‡	5.8-8.5
Mean RBC volume (fl)	70 ± 6	71 ± 6	70 ± 6	70 ± 6	62-77
MCHC (g/dL)	34.0 ± 0.8	33.9 ± 1.1	34.1 ± 0.6	33.9 ± 0.9	33.0-36.1
Reticulocytes ($\times 10^9$/L)	**45.3 ± 40.0**	**22.3 ± 14.1**	**27.0 ± 12.0**	**10.4 ± 5.7**§‖	2.9-4.2

Note: Data shown as mean ± SD. Bold font indicates mean value outside reference range.
‡P < .05, §P < .005, versus time of admission for that group, by paired Student's t-test.
‖P < .05, versus Placebo at same timepoint, by unpaired Student's t-test.
[a]0.1 mL/kg sterile 0.9% saline, IV, q12h, n = 8.
[b]CDP-choline (5 mg/kg) in 0.1 mL/kg sterile 0.9% saline/kg, IV, q12h, n = 10.

Figure 4B). The mean A-a gradient in the drug-treated group decreased from 52 mmHg at the time of enrollment to 40 mmHg at 24 hours and 31 mmHg at 48 hours. In contrast, P:F ratios and A-a gradients did not improve significantly in placebo-treated dogs. In these animals, the mean P:F ratio increased from 322 mmHg at the time of enrollment to 343 mmHg at 48 hours, whereas the mean A-a gradient decreased from 47 mmHg at the time of enrollment to 39 mmHg at 48 hours (Table 3).

3.4 | Intravenous CDP-choline prevented platelet consumption in dogs with aspiration pneumonia

At the time of admission, dogs with aspiration pneumonia exhibited modest lymphopenia and prominent neutrophilia (Table 4), although differential leukocyte counts and erythrocyte parameters did not differ significantly between dogs assigned to the placebo and treatment groups at this time. Mononuclear leukocyte counts did not change

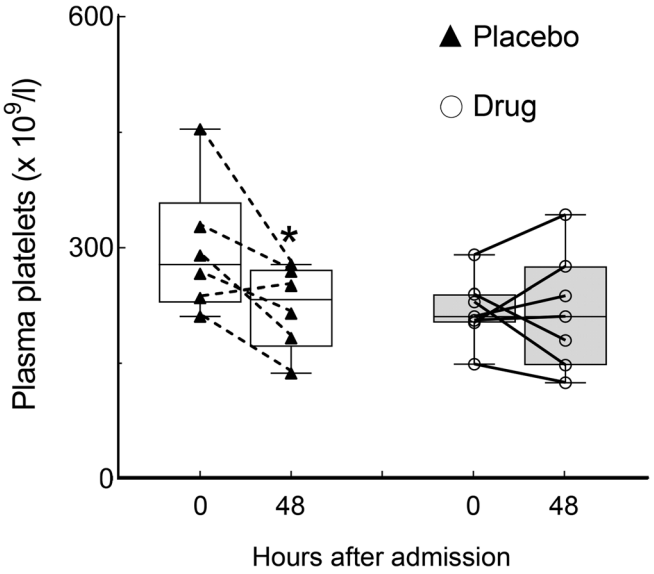

FIGURE 5 Intravenous cytidine diphosphocholine (CDP)-choline prevents platelet consumption in dogs with aspiration pneumonia. Effect of treating dogs with aspiration pneumonia with placebo (0.1 mL/kg sterile 0.9% saline, i.v.) or drug (CDP-choline, 5 mg/kg in 0.1 mL/kg sterile 0.9% saline, i.v.) every 12 hours over the first 48 hours after admission to the OSUVMC Small Animal intensive care unit on plasma platelet counts ($\times 10^6$/mL). n = 6 placebo-treated dogs and 7 drug-treated dogs. Data in box represent first quartile, median, and third quartile for each treatment group. Whiskers indicate highest and lowest sample values within the group. Data for each individual dog are linked over time by connecting lines. All data were analyzed by paired ANOVA with a post hoc Tukey-Kramer multiple comparison post-test. *P < .05, versus time 0 in same treatment group

significantly at 48 hours in placebo-treated dogs and were not affected by CDP-choline administration. However, neutrophil counts increased at 48 hours in both groups. Hematocrit and erythrocyte counts were low in both groups at 48 hours, but the absence of a change in mean cell hemoglobin concentration suggests that this finding was a consequence of hemodilution secondary to IV fluid administration rather than being indicative of anemia (Table 4). Interestingly, platelet and reticulocyte counts decreased significantly in placebo-treated but not in CDP-choline-treated dogs at 48 hours (Figure 5 and Table 4) and reticulocyte counts were significantly lower in the drug-treated group than in placebo controls at this timepoint.

3.5 | Intravenous CDP-choline had no significant systemic impact on function of other organs

In general, any abnormalities in plasma electrolyte (Table 5) and glucose concentrations (Table 6), as well as indices of hepatic and renal function (Table 6) related to the presence of aspiration pneumonia were modest at the time of admission, and results did not differ significantly between treatment groups. Despite within-group differences that reached statistical significance at 48 hours (including a decrease in plasma albumin concentration likely caused by hemodilution), no clinically relevant differences were observed between placebo- and CDP-choline-treated dogs at this timepoint.

4 | DISCUSSION

Aspiration pneumonia accounted for 0.5% of the cases admitted to OSUVMC Small Animal ICU over the last 15 years and remains the most common risk factor for development of VetARDS in dogs.[3] The diagnostic criteria for VetARDS in dogs are similar to those in the original AECC definition of ARDS in humans.[2] However, mortality rates are far higher (84%-100%), primarily because owners often elect euthanasia instead of treatment.[3] For this reason, our clinical trial was limited to dogs with mild VetARDS caused by aspiration pneumonia and not requiring mechanical ventilation. We evaluated S_pO_2, P:F ratios, and A-a gradients in room air, the last of which is more relevant in spontaneously breathing pneumonia patients.[23] Despite the small size of our trial, we found that addition of CDP-choline to the treatment regimen significantly improved gas exchange by 48 hours, as measured by all 3 parameters (SpO2, P:F ratio, A-a gradient): these effects could not be ascribed to differences in antibiotic usage or

TABLE 5 Plasma electrolyte concentration upon admission and at 48 hours in intensive care unit-admitted dogs, randomized to receive IV saline placebo or IV CDP-choline for the treatment of aspiration pneumonia.

	Placebo[a]		Drug[b]		
	Admission	48 hours	Admission	48 hours	Reference
Sodium (mEq/L)	143.8 ± 1.7	148.3 ± 2.3[§]	146.7 ± 2.5	147.7 ± 3.8	143-153
Potassium (mEq/L)	**3.9 ± 0.6**	**4.1 ± 0.6**	**4.1 ± 0.3**	4.5 ± 0.3[‡]	4.2-5.4
Chloride (mEq/L)	111 ± 3	110 ± 6	112 ± 3	112 ± 3	109-120
Bicarbonate (mmol/L)	18.0 ± 1.7	22.0 ± 2.7[‡]	18.5 ± 3.8	22.1 ± 4.1[‡]	16-25
Calcium (mg/dL)	**9.0 ± 1.7**	**9.2 ± 0.6**	9.4 ± 1.9	9.3 ± 0.6	9.3-11.6
Phosphorus (mg/dL)	3.8 ± 1.7	4.5 ± 1.1	4.1 ± 1.3	4.8 ± 1.3	3.2-8.1
Osmolality (mOsm/kg)	288 ± 8	295 ± 3	296 ± 3	292 ± 6	285-304

Note: Data shown as mean ± SD. Bold font indicates mean value outside reference range.
[‡]P < .05, [§]P < .005, versus time of admission for that group, by paired Student's t-test.
[a]0.1 mL/kg sterile 0.9% saline, IV, q12h, n = 8.
[b]CDP-choline (5 mg/kg) in 0.1 mL/kg sterile 0.9% saline/kg, IV, q12h, n = 10.

TABLE 6 Serum glucose concentrations and liver and kidney function test results upon admission and at 48 hours in intensive care unit-admitted dogs, randomized to receive IV saline placebo or IV CDP-choline for the treatment of aspiration pneumonia.

	Placebo[a]		Drug[b]		
	Admission	48 hours	Admission	48 hours	Reference
Glucose (mg/dL)	103 ± 11.3	113 ± 23	103 ± 25	103 ± 16	75-125
BUN (mg/dL)	11.1 ± 3.1	8.8 ± 2.8	13.2 ± 6.0	10.2 ± 7.6[§]	5-20
Creatinine (mg/dL)	0.76 ± 0.14	**0.58 ± 0.25**	0.88 ± 0.35	0.67 ± 0.31	0.6-1.6
Alanine aminotransferase (IU/L)	**73 ± 71**	**59 ± 52**	51 ± 44	54 ± 38	10-55
Aspartate aminotransferase (IU/L)	27 ± 8.5	**47 ± 17**[§]	**51 ± 28**[‖‖]	**80 ± 82**	12-40
Alkaline phosphatase (IU/L)	**164 ± 76**	**356 ± 352**[§]	**131 ± 70**	**179 ± 89**	15-120
Creatine kinase (IU/L)	173 ± 99	305 ± 192	**442 ± 357**[‖‖]	**827 ± 818**	50-400
Total bilirubin (mg/dL)	0.11 ± 0.11	0.33 ± 0.62	0.13 ± 0.09	0.1 ± 0.03	0.1-0.4
Total protein (g/dL)	5.9 ± 0.8	5.2 ± 0.6[‡]	5.8 ± 0.9	5.1 ± 0.9[‡]	5.1-7.1
Albumin (g/dL)	3.2 ± 0.3	**2.6 ± 0.3**[§]	3.3 ± 0.6	**2.5 ± 0.3**[‖]	2.9-4.2
Globulin (g/dL)	2.8 ± 0.6	2.6 ± 0.3	2.5 ± 0.6[‖]	2.6 ± 0.6	2.2-2.9

Note: Data shown as mean ± SD. Bold font indicates mean value outside reference range.
[‡]$P < .05$, [§]$P < .005$, [‖]$P < .001$, versus time of admission for that group, by paired Student's *t*-test.
[‖‖]$P < .05$, versus Placebo at same timepoint, by unpaired Student's *t*-test.
[a]0.1 mL/kg sterile 0.9% saline, IV, q12h, n = 8.
[b]CDP-choline (5 mg/kg) in 0.1 mL/kg sterile 0.9% saline/kg, IV, q12h, n = 10.

other therapeutic interventions between groups because such differences were not present. Significant improvements in P:F ratios and A-a gradients were evident within the first 24 hours after ICU admission, although the effect of CDP-choline on S_pO_2 at this timepoint was not significant ($P < .1$). In addition, CDP-choline appeared to have a platelet-sparing effect, which was comparable to that reported previously.[24,25] Importantly, we found no evidence of adverse systemic effects in CDP-choline-treated dogs. To our knowledge, our study represents the first time that a novel ARDS treatment identified in mice[12] has been evaluated for efficacy in larger animals with spontaneously occurring (as opposed to experimentally induced) lung injury under clinical conditions. Our study demonstrates the feasibility of such an approach, and also suggests that CDP-choline should be further investigated as a potentially a safe and effective adjunctive treatment in dogs with mild VetARDS caused by aspiration pneumonia.

We found CDP-choline treatment increased mean P:F ratios in dogs with aspiration pneumonia by >60 mmHg over the first 24 hours. In comparison, recent meta-analyses showed that treatment of human ARDS patients using inhaled nitric oxide only increased P:F ratios by a mean of 20 mmHg over the same time period,[26] although the inhaled prostaglandin epoprostenol increased P:F ratios by a mean of 54 mmHg within 1 day.[27] In the clinical trial, therapeutic versus prophylactic anticoagulation for severe COVID-19: a randomized phase II clinical trial (HESACOVID), a 46 mmHg increase in P:F ratios over 7 days of treatment with the anticoagulant enoxaparin was associated with a significant increase in ventilator-free days.[28] Similarly, treatment of coronavirus disease 2019 (COVID-19) patients using the Janus kinase (JAK) inhibitor baricitinib increased P:F ratios by 49 mmHg over 4 days and also significantly decreased mortality.[29] Likewise, proning maneuvers temporarily improved P:F ratios by a mean of 44 mmHg in 34 mechanically ventilated COVID-19 patients, although the effect on outcomes was not determined.[30] Hence, the effects of CDP-choline on gas exchange are comparable to those of several other potential adjunct treatments undergoing clinical trials in human ARDS or COVID-19 patients. Physiologic improvements in gas exchange do not necessarily translate into decreases in mortality in ARDS patients.[31] However, oxygenation frequently is used to stratify ARDS severity,[32] and lower P:F ratios have been associated clinically with more lung inhomogeneity and increased lung water on computed tomography scans,[33] as well as with poorer responses to low tidal volume ventilation.[34] Moreover, in a secondary analysis of data from the LOVS and ExPress trials, it was found that an increase in P:F ratio of >25 mmHg after prone positioning was strongly associated with a 20% decrease in mortality rate.[35]

In addition to its marked impact on oxygenation, CDP-choline has many advantageous characteristics as a VetARDS drug. It is an inexpensive, stable, and exceptionally safe natural metabolite that can be administered PO and parenterally.[36] It is well tolerated in both dogs and humans at dosages >1 g/kg/day and adverse effects are rare (<1:10 000).[14,37] Cytidine diphosphocholine has high solubility and bioavailability after parenteral administration and does not need to be instilled directly into the lungs. Consequently, issues commonly associated with intrapulmonary drug administration in ARDS, such as heterogeneity of distribution in the injured lung[38] and induction of transient hypoxemia[39] can be avoided. Moreover, CDP-choline that is not incorporated into phospholipids is rapidly metabolized into cytidine and choline, and ultimately excreted in exhaled air and in urine.[37] Several phase III clinical trials of CDP-choline have been conducted in humans (primarily for neurologic disorders[37]) and the pharmacokinetics of PO and IV CDP-choline are well understood in both dogs and

humans and highly comparable.[40] For example, in healthy adult humans, >99% of a single PO dose of 300 mg of ^{14}C-labeled CDP-choline was absorbed, which resulted in 2 peaks in plasma radioactivity at 1 and 24 hours post-dose, the second peak being larger.[41] After 5 days, 16% of the administered dose had been recovered, suggesting that the remainder had been incorporated into tissues with high efficiency or was available for biosynthetic and biodegradative pathways. Finally, CDP-choline is markedly anti-inflammatory but lacks the adverse systemic effects associated with corticosteroids.[12,42]

Although the mechanism by which CDP-choline improves gas exchange is not clear, we hypothesize that it is associated with its anti-inflammatory effects. Treatment with CDP-choline did not significantly alter peripheral leukocyte counts in dogs, but it was not possible to measure bronchoalveolar lavage fluid infiltrates and thus we do not yet know the impact of CDP-choline on pulmonary inflammation in dogs. Likewise, it is not clear why LPNs have anti-inflammatory properties, but these properties could result from changes in recruitment signals (eg, from ATII cells), microvascular permeability,[42] leukocyte function (as a direct result of LPN uptake by these cells) or some combination of these. Finally, it is unknown why CDP-choline acts so rapidly. Significant improvements in gas exchange were detected in some dogs within 12 hours after enrollment. Data in mice indicate that CDP-choline treatment reverses IAV-induced inhibition of alveolar fluid clearance, which would decrease the diffusion distance for O_2 across the alveolocapillary membrane and could improve ventilation/perfusion matching.[12]

Our study had some limitations, the most important of which is that the small group sizes, heterogeneity of the patient population (differences in breed, size, and sex), and relatively low disease severity decreased statistical power. We attempted to compensate for these factors by following CDP-choline treatment effects over time in individual dogs and comparing patient-specific outcome measures within as well as between treatment groups. However, difficulty in obtaining arterial blood samples from all dogs at all timepoints because of patient compliance issues further decreased group sizes for these analyses. An additional limitation is that the CDP-choline treatment regimen was empirical and based on our study of mice.[12] It is possible that higher, more frequent, or even continuous dosing would provide more clinical benefit, particularly if other LPNs were added to the treatment regimen. Despite these limitations, we still were able to identify significant improvements in gas exchange in CDP-choline-treated dogs in the first 48 hours after enrollment for mild VetARDS. Although LPNs may be less effective in sicker dogs, our data from mice argue against this conclusion because these data they show that LPNs (including CDP-choline at the same dose as used in our current study) can attenuate or even prevent hypoxemia in mice with severe ARDS.[12]

In conclusion, we determined that administration of CDP-choline to dogs with spontaneously occurring mild VetARDS caused by aspiration pneumonia rapidly and significantly improved gas exchange with minimal adverse effects. These findings suggest that additional clinical trials should be performed to determine whether CDP-choline and other LPNs should be considered as potential adjunctive treatments in dogs with VetARDS to improve the currently poor survival rate for this condition.

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CONFLICT OF INTEREST

Ian C. Davis is the inventor on a patent owned by The Ohio State University that is related to cytidine diphosphocholine technology and has received licensing fees from a commercial entity. However, that entity is no longer the licensee and did not provide any financial or in-kind support for these studies or have any editorial or other input to this manuscript. All authors confirm that the manuscript is accurate and transparent account of the study being reported and that no important aspects of the study have been omitted.

OFF-LABEL ANTIMICROBIAL DECLARATION

The authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the IACUC (protocol #2017A000085: Liponucleotides For Treatment Of Canine ARDS) and the Veterinary Clinical Research Oversight Committee at The Ohio State University.

HUMAN ETHICS APPROVAL DECLARATION

The Authors declare human ethics approval was not needed for this study.

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