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PII: S0034-5288(21)00303-9

DOI: https://doi.org/10.1016/j.rvsc.2021.10.010

Reference: YRVSC 4472

To appear in: *Research in Veterinary Science*

Received date: 1 September 2020

Revised date: 10 June 2021

Accepted date: 5 October 2021

Please cite this article as: M. Kocaturk, Z. Yilmaz, M. Cansev, et al., Choline or CDP-choline restores hypotension and improves myocardial and respiratory functions in dogs with experimentally – Induced endotoxic shock, *Research in Veterinary Science* (2021), https://doi.org/10.1016/j.rvsc.2021.10.010

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Choline or CDP-choline restores hypotension and improves myocardial and respiratory functions in dogs with experimentally - induced endotoxic shock

Meric Kocaturk[1*], Zeki Yilmaz[1], Mehmet Cansev[2], Yesim Ozarda[3], Jose Joaquin Ceron[4], Ali Buturak[5], Ismail H. Ulus[6]

1 Assoc. Prof. Meric Kocaturk; Prof. Dr. Zeki Yilmaz, Department of Internal Medicine, Faculty of Veterinary Medicine, Bursa Uludag University, 16059 Bursa, Turkey. MK: merick@uludag.edu.tr; ZY: zyilmaz@uludag.edu.tr

2 Prof. Dr., Department of Pharmacology, Bursa Uludag University School of Medicine, Bursa, Turkey. MC: mcansev@uludag.edu.tr

3 Prof. Dr., Department of Medical Biochemistry, Istanbul Health and Technology University, School of Medicine, Istanbul, Turkey. YO: yesim.ozarda@istun.edu.tr

4 Prof. Dr., Interdisciplinary Laboratory of Clinical Analysis, Interlab-UMU, Regional Campus of International Excellence "Campus Mare Nostrum", University of Murcia, Espinardo, Murcia 30100, Spain. JJC: jjceron@um.es

5 Assoc. Prof., Liv Hospital Vadi Istanbul, Cardiology Clinic, Istanbul, Turkey. AB: alibuturak@yahoo.com

6 Prof. Dr., Ismail H. Ulus, Acibadem Mehmet Ali Aydinlar University Medical School, Department of Pharmacology. Istanbul, Turkey. IHU: ihakki.ulus@acibadem.edu.tr

* Corresponding author:

Meric KOCATURK

E-address: merick@uludag.edu.tr, ORCID: 26 0000-0002-2849-1222

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ABSTRACT

Endotoxin shock is associated with severe impairments in cardiovascular and respiratory functions. We showed previously that choline or cytidine-5'-diphosphocholine (CDP-choline) provides beneficial effects in experimental endotoxin shock in dogs. The objective of the present study was to determine the effects of choline or CDP-choline on endotoxin-induced cardiovascular and respiratory dysfunctions.

Dogs were treated intravenously (i.v.) with saline or endotoxin (LPS, 0.1 mg/kg) 5 min before i.v. infusion of saline, choline (20 mg/kg) or CDP-choline (70 mg/kg). Blood pressure, cardiac rate, myocardial and left ventricular functions, respiratory rate, blood gases, serum electrolytes and cardiac injury markers were determined before and at 0.5-48h after endotoxin. Plasma tumor necrosis factor alpha (TNF-α), high mobility group box-1 (HMGB1), catecholamine and nitric oxide (NO) levels were measured 2h and 24h after the treatments.

Endotoxin caused immediate and sustained reductions in blood pressure, cardiac output, pO_2 and pH; changes in left ventricular functions, structure and volume parameters; and elevations in heart rate, respiratory rate, pCO2 and serum electrolytes (Na, K, Cl, Ca and P). Endotoxin also resulted in elevations in blood levels of cardiac injury markers, TNF-α, HMGB1, catecholamine and NO. In choline- or CDP-choline-treated dogs, all endotoxin effects were much smaller in magnitude and shorter in duration than observed values in controls.

These data show that treatment with choline or CDP-choline improves functions of cardiovascular and respiratory systems in experimental endotoxemia and suggest that they may be useful in treatment of endotoxin shock in clinical setting.

Key words: Choline, endotoxemia, sepsis, cardiopulmonary function, dogs

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1. Introduction

Sepsis causes life-threatening organ dysfunction mediated by dysregulated host response to infection (Hoffmann and Behnes, 2016). Septic shock is a subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to result in substantial increases in mortality (Hoffmann and Behnes, 2016). Lipopolysaccharide (LPS), commonly known as endotoxin, is a prominent cell wall component of gram-negative bacteria (Raetz and Witfield, 2002; Alexander and Rietschel, 2001) and one of the main triggers for dysregulated host responses, tissue injury, organ dysfunction, sepsis and septic shock via interacting with the toll-like receptor (TLR)-4 (Raetz and Witfield, 2002; Alexander and Rietschel, 2001; Gnauck et al, 2016; Poltorak et al, 1998; Park et al, 2009). LPS binds to TLR4 to cause an NF-κB-dependent dysregulated host response cascade resulting in excessive production of inflammatory and pro-inflammatory cytokines [i.e., tumor necrosis factor alpha (TNF-α) and interleukin 6 (IL-6)] and other mediators. Thus, therapeutic strategies in sepsis/endotoxemia involve elimination of LPS (endotoxins) source (by using effective antibiotics), suppression of excessive production of toxic mediators in LPS-sensitive cells, and/or blockade of deleterious actions of these inflammatory and pro-inflammatory mediators.

Cholinergic system modulates functions of the LPS-responsive immune cells (Bernik et al, 2002; Hoover, 2017; Rosas-Ballina and Tracey, 2019; Kanashiro et al, 2017), and elevation in cholinergic tone induces beneficial effects in experimental endotoxin/septic shock model (Rosas-Ballina and Tracey, 2019; Kanashiro et al, 2017; Rosas-Ballina et al, 2008; Pavloc et al, 2007; Parrish et al, 2008; Ilcol et al, 2005; Ilcol et al, 2009). Rates of synthesis and release of acetylcholine can be affected by the levels of its precursor, choline (Ulus and Wurtman, 1976; Ulus et al, 1977; Meyer and Baker et al, 1986; Ulus et al, 2006;

Johnson et al, 1992; Buyukuysal et al, 1995). Choline is also an agonist for cholinergic receptors (Ulus et al, 1988) and treatment with choline increases cholinergic transmission (Ulus and Wurtman, 1976; Ulus et al, 1977; Meyer and Baker et al, 1986; Ulus et al, 2006; Johnson et al, 1992; Buyukuysal et al, 1995) and produces biological effects in cholinergic nature (Savci et al, 2002; Ilcol et al, 2002; Ilcol et al, 2003). In consistency with the results of previous studies (Bernik et al, 2002; Hoover, 2017; Rosas-Ballina and Tracey, 2019; Kanashiro et al., 2017; Rosas-Ballina et al, 2008; Pavloc et al, 2007; Parrish et al, 2008), our studies in dogs (Ilcol et al, 2005 and 2009; Yilmaz et al, 2006, 2010 and 2016; Tvarijonaviciute et al, 2012; Kocaturk et al, 2016), calves (Yilmaz et al, 2016; Eralp Inan et al, 2019), and rats (Ilcol et al, 2009) showed choline-induced elevation in cholinergic activity, which in turn mediated the body's response to bacterial endotoxin. Accordingly, choline treatment reduces endotoxin-induced multiple organ injury (Ilcol et al, 2005), improves platelet functions (Yilmaz et al, 2006) and coagulation abnormalities (Yilmaz et al, 2010), prevents disseminated intravascular coagulation (Yilmaz et al, 2010), attenuates the elevation in circulating TNF-α (Parrish et al, 2008; Ilcol et al, 2005) and lipids levels (Ilcol et al, 2009), reduces the alterations in circulating blood cells counts (Yilmaz et al, 2006) and in some specific serum proteins (Tvarijonaviciute et al, 2012; Kocaturk et al, 2016; Yilmaz et al, 2016; Eralp Inan 2019) and enhances survival rate (Parrish et al, 2008; Ilcol et al, 2005). In addition to choline itself, treatment with cytidine-5-diphosphocholine (CDP-choline) is also found very effective in preventing endotoxin-induced platelet dysfunctions (Yilmaz et al, 2006), coagulation abnormalities (Yilmaz et al, 2010) and disseminated intravascular coagulation (Yilmaz et al, 2010) in dogs.

Endotoxin is known to cause myocardial and lung injuries, respiratory and cardiovascular dysfunctions associated with profound and long-lasting hypotension (endotoxin shock) in dogs (Evans et al, 1984; Natanson et al, 1984; Farfia et al 1998; Batmaz et al, 2003). Based

on the described beneficial actions of choline and CDP-choline in experimental endotoxemia, we hypothesized that choline and/or CDP-choline might also prove effective for restoring cardiovascular and respiratory dysfunctions induced by endotoxin. This study was designed to test this hypothesis in our experimental endotoxin shock model in dogs (Ilcol et al, 2005; Ilcol et al, 2009; Yilmaz et al, 2006; Yilmaz et al, 2010; Tvarijonaviciute et al, 2012). We report here that choline or CDP-choline improves cardiovascular and respiratory dysfunctions induced by endotoxin.

2. Material and Methods

2.1.Dogs

Thirty-six healthy (based on the routine clinical, hematological and serum biochemistry findings) male mongrel dogs (1-6 years old and 20-30 kg) were used in the present study. Dogs were hospitalized for 10 days before the initiation of experiments in the Veterinary Teaching Hospital (Bursa Uludag University – Bursa / Turkey). Water was provided *ad libidum*, and a standardized pellet diet was fed once a day in this period.

All procedures in the study were performed in accordance with The Governmental Rules and Guides. The experimental protocol was approved by the Animal Care and Use Committee of Bursa Uludag University, Bursa–Turkey (10.02.2009, 2009 – 01 / 08).

2.2.Groups and Experiments

Thirty-six dogs were randomized equally into six groups. Dogs in the first, second and the third groups received 0.2 mL/kg of sterile saline solution (0.9 % NaCl) via intravenous (i.v.) route, while dogs in the fourth, fifth and sixth groups received 0.2 ml/kg of saline containing 0.1 mg/kg of endotoxin via i.v. route. Five minutes after the first i.v. injection, dogs received a second i.v. injection of 0.2 mL/kg saline (first and fourth groups), or 0.2

mL/kg saline containing either 20 mg/kg of choline (second and fifth groups) or 70 mg/kg of CDP-choline (third and sixth groups). Groups were designated as: Saline+Saline (first group), Saline+Choline (second group), Saline+CDP-Choline (third group), Endotoxin+Saline (fourth group), Endotoxin+Choline (fifth group) and Endotoxin+CDP-Choline (sixth group).

The dose of endotoxin (0.1 mg/kg), choline (20 mg/kg) and CDP-Choline (70 mg/kg) and treatment regimens were selected from our previous studies in dogs (Ilcol et al, 2005; Ilcol et al, 2009; Yilmaz et al, 2006; Yilmaz et al, 2010; Tvarijonaviciute et al, 2012), according to the recommendation of the Animal Care and Use Committee of Bursa Uludag University (Bursa, Turkey).

2.3. Measurements

All dogs were monitored by clinical and laboratory examinations to determine alterations in cardiovascular and respiratory functions for 48 h. Blood samples were collected into vacutainer tubes (Becton Dickinson Vacutainer System, NJ, USA) before (0 time) and at 0.5, 1, 2, 4, 8, 12, 24, and 48 h after the treatments.

2.4.Blood pressure measurements

Systolic (SAP), diastolic (DAP) and mean arterial blood pressures (MAP) were measured non-invasively from right antebrachial artery with the dogs in sternal recumbency (Vmed Vet-Dop2 Veterinary Doppler, USA), as reported earlier (Agudelo et al, 2016), at the blood sampling times.

2.5.Electrocardiographic (ECG) examination and measurements

ECG examinations were performed by the same investigator (M Kocaturk) with dogs in right lateral recumbency (3-chanel, veterinary ECG system, ECG-1103GW Vet, Carewell, China) on a suitable table. In addition to cardiac rhythm analysis, amplitudes of the ECG waves and the duration of the intervals were measured on lead II (50 mm/sec; 10 mm/mV), as performed in our previous studies (Agudelo et al, 2016),

2.6.Echocardiographic examinations and measurements

Echocardiographic examinations, which were performed by two experts (Z Yilmaz and M Kocaturk), included two-dimensional (2D), M-mode, color flow imaging, and spectral Doppler measurements (Caris Plus, Esoate, Florence, Italy) with standard imaging techniques (right parasternal short and long axis views and left parasternal apical 4-5 chamber view), for which 2.5–5 and 5–7.5 MHz phased-array cardiac transducers were used, as suggested (Boon, 2011). The dogs were gently restrained, without sedation, in right and left lateral recumbency on a table suitable for transthoracic echocardiographic examinations as performed in our previous study (Kocaturk et al; 2010).

Left ventricular (LV) related geometric [LVDD (left ventricular diastolic diameter, LVSD (left ventricular diastolic diameter), LVPWDD (left ventricular posterior wall diastolic diameter), LVPWSD (left ventricular posterior wall systolic diameter), IVSDD (interventricular septal diastolic diameter) and IVSSD (interventricular septal systolic diameter)], and volumetric parameters [LVDV (left ventricular diastolic volume) and LVSV (left ventricular systolic volume)] were measured by 2D M-mode on the right parasternal long axis 4 chamber views. Stroke volume (SV) was estimated automatically at the same picture by echo machine. Cardiac output (CO; mL) was calculated manually by multiplying SV (cm^3) and heart rate (/min), observed automatically from the ECG.

LV systolic and diastolic functions were measured by Pulse Wave (PW) Doppler imaging. For this purpose, aortic (Ao) blood flows were evaluated on the left parasternal apical 5 chamber view, and systolic time intervals such as ejection time (ET), pre-ejection period (PEP), and PEP/ET ratio were measured. Mitral inflow maximal velocities of E (left ventricular relaxation in early diastole) and A waves (late diastole caused by atrial contraction) and their ratio (mitral E/A) on the left apical 4-chamber view were measured to assess LV diastolic function, as reported in our previous study (Kocaturk et al, 2010).

The LV Tei index (myocardial performance index - MPI), representing overall Doppler composite index for both systolic and diastolic ventricular function, was calculated by PW Doppler, and MPI was defined as the sum of isovolumic contraction time (ICT) and isovolumic relaxation time (IVRT) divided by the ET on left apical 5-chamber view, as described previously (Kocaturk et al, 2010; Chetboul et al, 2016). Briefly, on left parasternal apical 5 chamber view, from the transmitral PW Doppler, the time interval between the end of mitral filling and the onset of mitral filling was determined ("a"). From transaortic PW Doppler, the time interval from the beginning of aortic flow to the end of aortic flow was determined ("b" or LVET). The MPI was calculated three times consecutively and its arithmetic mean was used for each dog, as reported in our previous article (Kocaturk et al, 2010), by using the formula: MPI = (a–b)/b.

2.7.Blood gas analysis

Blood (venous) gas analysis was performed, within 5 min after sampling from cephalic veins, by use of automatic blood gas analyzer (IRMA TruPoint, ITC, NJ – USA). Partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2), pH, HCO3 (standard and active), base excess, extracellular base excess, O_2 saturation and total carbon dioxide concentration ($CtCO_2$) were evaluated.

2.8.Biochemical analysis

Serum cardiac injury biomarkers creatinine kinase myocardial isoenzyme (CK-MB), beta-hydroxybutyric acid dehydrogenase (BHBD), myoglobin and cardiac troponin I (cTnI) and electrolytes (Na, K, Mg, Cl, Ca and P) were measured by the use of automatic analyzer (Architect ci8200; Abbott, GmbH Wiesbaden, Germany), according to manufacturers' protocols for each assay.

Serum High mobility group box – 1 (HMGB1) protein was measured by using commercially available assay kit (Life Science HMGB1 ELISA assay kits, Wuhan USCN Business Company, China) according to manufacturer's protocols.

Serum TNF-α was measured by using commercially available ELISA kit (TNF-α assay kit, BioSource Europe A.S., Nivelles, Belgium), as reported earlier (Ilcol et al, 2005).

Serum nitrate/nitrite was measured by colorimetric assay kit (RD system, Minneapolis, MN, USA), as reported earlier (Yilmaz et al, 2008).

Plasma catecholamine was measured by radioimmunoassay using a commercially available assay kit (Biosource, Europe, Nivelles, Belgium), as reported earlier (Yilmaz et al, 2008).

2.9.Chemicals

Choline chloride, CDP-choline disodium and endotoxin [*lipopolysaccacharide* (LPS); *E. coli,* 055: B5 serotype, purity>97%] were purchased from Sigma Chemical Co., St. Louis Missouri, USA).

2.10. Data analysis

Data were expressed as mean ± standard error of the mean (SEM) and analyzed by one-way or two-way analysis of variance (ANOVA) with repeated measures, followed by post-hoc Tukey test for multiple-comparisons (SigmaPlot Version 11; Systat Software GmbH,

D-40699, Erkrath, Germany). Comparisons between two groups were made using *Student's t-test*. *P* value less than 0.05 was considered significant.

3. Results

3.1.Effects of choline or CDP-choline on non-invasive blood pressure measurements in control and endotoxemic dogs

The baseline values of the SAP, DAP and MAP in dogs (n=36) were 130 ± 2 mm Hg, 97 ± 2, mm Hg and 112 ± 2 mm Hg, respectively.

In the control groups, saline treatment failed to alter these blood pressure parameters significantly [F(7,35) = 0.270, P = 0.969] and these parameters remained more or less the same during the 48-h observation period (Fig. 1A-C.). Statistically significant changes were observed with regard to non-invasive blood pressure measurements in dogs that did not receive endotoxin. Intravenous infusion of choline (20 mg/kg) increased SAP by 17 ± 5 mmHg (P<0.05, paired *t-test*) compared to pre-treatment values at 0.5 h (Fig. 1A.). At all other time points, blood pressure values were not different from baseline and/or respective control (saline + saline) values. Intravenous infusion of CDP-choline (70 mg/kg) increased SAP [F(1,62) =12.38, P<0.01] and MAP [F(1,62) =2.38, P<0.05] for 2 h. Blood pressure values returned to their baseline at 2 h, and remained more or less stable during the observation period (Fig. 1A., C. and E.).

In the test groups that received endotoxin along with saline, choline or CDP-choline, blood pressure parameters showed statistically significant differences within and between groups throughout the study. SAP [F(7,30) =7.52, P<0.001], DAP [F(7,30) =4.45, P<0.001] and MAP [F(7,30) =4.32, P<0.001] decreased significantly in response to endotoxin (0.1 mg/kg) in saline-treated dogs (Fig. 1B., D. and F.). As seen in Fig. 1., endotoxin-induced

decreases in blood pressure were prevented or attenuated by choline or CDP-choline. Statistical analysis by two-way repeated measures ANOVA revealed significant effect of choline [$F(1,63) = 2.89$, $P<0.05$], [$F(1,69) = 5.35$, $P<0.001$] and [$F(1,69) = 8.56$, $P<0.001$], or CDP-choline [$F(1,69) = 12.38$, $P<0.001$], [$F(1,110) = 14.35$, $p<0.001$] and [$F(1,69) = 9.87$, $P<0.001$] on the endotoxin-induced changes in SAP, DAP and MAP, respectively. There were also significant choline-time and CDP-choline-time interactions on the endotoxin-induced changes in blood pressure variables.

3.2.Effect of choline or CDP-choline on heart rate in control and endotoxemic dogs

Baseline heart rate was 113 ± 3 bpm (n = 36). In the control groups that did not receive endotoxin, heart rate did not change significantly from ~~the initial~~ baseline values during 48 h observation period in dogs treated either with saline + saline [$F(7,35) = 0.761$, $P = 0.638$] or saline + CDP-choline [$F(7,35) = 0.29$, $P = 0.11$] (Fig. 2A.). Heart rate decreased by 27 ± 6 and 19 ± 6 bpm ($P<0.05$, paired t test) from baseline values at 0.5 h and 1 h after choline treatment, respectively (Fig. 2A.).

In endotoxin-receiving groups, heart rate increased significantly [$F(7,31) = 9.95$, $P<0.001$] for 48 h in response to endotoxin (Fig. 2A.). This increase was only observed during 0.5-4 h but disappeared at 12-48 h after endotoxin in CDP-choline-treated dogs (Fig. 2B.). In choline-treated dogs, heart rate decreased at 0.5 and 1 h ($P<0.05$) and remained more or less stable approximating to baseline values at 2-48 h after endotoxin (Fig. 2B.). Statistical analysis by two-way repeated measures ANOVA revealed significant effect of choline [$F(1,64) = 43.34$, $P<0.001$] or CDP-choline [$F(1,68) = 25.00$, $P<0.001$] on the endotoxin-induced changes in heart rates, and significant choline-time [$F(7,64) = 5.41$, $P<0.001$] and CDP-choline-time [$F(7,68) = 9.17.15$, $P<0.001$] interactions.

In CDP-choline-treated animals, the endotoxin-induced increases in heart rate were attenuated during the first 4 h, and blocked between 12-48 h after endotoxin treatment (Fig. 2B.). Statistical analysis by two way repeated measures ANOVA revealed significant effect for choline [$F(1,68) = 25.00$; $P<0.001$], time [$F(7,68) = 25.65$; $P<0.001$] and choline-time interaction [$F(7,68) = 9.17$; $P<0.001$] on endotoxin action.

3.3.Effects of choline or CDP-choline on electrocardiographic (ECG) parameters in control and endotoxemic dogs

Saline-treated control dogs showed normal sinus rhythm and P-QRS-T configuration during the 4 h observation period. Choline caused sinus arrhythmia with a tendency to bradycardia and Wandering Sinus Pacemaker (WSP) for the first 2 h of the observation period and dogs returned to normal sinus rhythm 2 h after the treatments. CDP-choline did not alter sinus cardiac rhythm during 4 h observation period. Endotoxin caused multifocal atrial tachycardia (MAT) and sinus tachyarrhythmia which remained more or less stable for the 48 h observation period. Other ECG parameters (amplitudes of P, R, and R waves; and duration of P and QRS, and PR and QT intervals) did not show statistically significant changes during the study within and between groups (data not shown).

3.4. Effects of choline or CDP-choline on echocardiographic parameters in control and endotoxemic dogs

The baseline value of CO was 3780 ± 210 ml/min in all dogs (n=36) in the study. Within the control groups, CO remained more or less stable during 48 h observation period in saline-treated dogs (data not shown). CO (Fig. 3A) decreased slightly for 4 hours after choline (Fig. 3A.), and 2 hours after CDP-choline (Fig. 3A) treatments.

CO depressed greatly within 1 h after endotoxin, and showed gradual recovery form the decreased levels towards its baseline value within 24-48 hours after endotoxin treatment

(Fig. 3B). The endotoxin-induced decline in CO was attenuated by choline at 1-12 h and blocked at 24-48 h (Fig. 3B). The endotoxin-induced decline in CO was attenuated greatly at 0.5 h and 1 h and blocked totally at 2-48 h by CDP-choline.

LV related geometric (LVDD, LVSD, LVPWDD, LVPWSD, IVSDD and IVSSD) and volumetric parameters (LVDV and LVSV) were not changed after either choline or CDP-choline treatment alone (data not shown). In saline-treated control dogs, endotoxin increased LVSD, LVPWDD, LVPWSD, IVSDD and IVSSD at 2-48 h (Table 2). Treatment with either choline or CDP-choline blocked the endotoxin-induced changes in these parameters (Table 2). LVDD decreased by endotoxin for 4 h, and this decline was blocked either with choline or CDP-choline treatment. Endotoxin decreased LVDV and LVSV at 2-24 h after treatment, and these declines were blocked either with choline or CDP-choline treatment (Table 2).

With regard to systolic time intervals, in saline- or choline-treated dogs, PEP value did not differ significantly throughout the study, but CDP-choline per se declined PEP value at 4-48 h ($P<0.01$) compared to baseline value. In endotoxin treated dogs, PEP value decreased from its baseline value of 486 ± 105 ms (baseline) to 291 ± 23 at 0.5 h ($P<0.001$) and 327 ± 24 ms at 1 h ($P<0.001$) and then reached to its baseline value at 48 h, whereas it did not change significantly for 48 h in choline- or CDP-choline-treated dogs receiving endotoxin. There was no statistically significant difference on ET in dogs treated with saline, choline or CDP-choline. Choline or CDP-choline treatment prevented the endotoxin-induced decreases in ET at 1-48 h. When PEP/ET was evaluated; saline, choline or CDP-choline treatment did not change the ratio for 48 h. Baseline PEP/ET value was decreased at 0.5 h ($P<0.001$) by endotoxin administration. Choline or CDP-choline treatment inhibited the endotoxin-induced decrease in the ratio of PEP and ET during the study.

Tei index did not show a statistically significant change except for the LPS group. Tei index increased statistically at 4 hours after LPS administration (1.8± 0.5; P<0.01) and then returned to baseline values (1,1 ± 0,2), whereas it did not change significantly during the study in choline- or CDP-choline-treated endotoxemic dogs (data not shown).

There were no statistically significant differences in mitral E/A ratio within and between control groups (saline, choline, or CDP-choline). Mitral E/A ratio decreased at 24 hours (P<0.01) after endotoxin, while choline and CDP-choline treatments prevented this reduction in endotoxin-treated dogs (data not shown).

1.1.Effects of choline or CDP-choline on cardiac injury markers in control and endotoxemic dogs

Baseline values for serum CK-MB, HBDH, myoglobin and cTnI were 140 ± 30 IU/L, 12.7 ± 1.5 IU/L, 0.34 ± 0.1 mg/dL and 0.05 ± 0.01 ng/mL, respectively. These cardiac injury markers did not change in saline+saline, saline+choline, saline+CDP-choline groups during the 48 h observation period (data not shown), while they were all increased by several folds in response to endotoxin (Table 2). Choline or CDP-choline attenuated the endotoxin-induced increases in these markers (Table 2).

1.2.Effect of choline or CDP-choline on respiratory rate in control and endotoxemic dogs

The baseline value of the respiratory rate was 16 ± 1 breaths per min. Saline or CDP-choline failed to alter the respiratory rate while this parameter increased markedly at 30 min and returned gradually to the baseline within the next 3 h after i.v. administration of choline (Fig. 4A.).

Respiratory rate increased markedly within 1h after endotoxin treatment and remained significantly high with a gradual decline during the 48-h observation period (Fig. 4B.). Endotoxin-induced increases in respiratory rate were attenuated at 1-4 h and blocked at 12-48 h period by choline or CDP-choline (Fig. 4B.). Statistical analysis by two way repeated measures ANOVA revealed significant effect for choline [$F(1,64) = 43.35$; $P<0.001$], time [$F(7,64) = 11.63$; $P<0.001$] and choline-time interaction [$F(7,64) = 5.41$; $P<0.001$] on endotoxin-induced changes in respiratory rate.

1.3.Effects of choline or CDP-choline on blood gases in control and endotoxemic dogs

Baseline values for venous partial pressure of oxygen (pO_2), partial pressure of carbon dioxide (pCO_2) and pH were 73.5 ± 2.1 mm Hg, 37.7 ± 1.2 mm Hg and 7.40 ± 0.01 in dogs, respectively. Saline or CDP-choline failed to alter pO_2, pCO_2, and pH and they remained more or less at baseline levels for 48-h observation period after treatments (Fig. 5A., C. and E.). After choline administration, pO_2 did not change (Fig. 5A.) but pCO_2 (Fig. 5C.) and pH (Fig. 5E.) values decreased significantly from their baseline values for the first 2 h after the treatment.

Endotoxin caused marked decrease in pO_2 (Fig. 5B.) and pH (Fig. 5F.), and about two-fold increase in pCO_2 values (Fig. 5D.) from their baseline values. Endotoxin-induced decline in pO_2 was attenuated by choline and blocked by CDP-choline (Fig. 5B.) administration. Choline or CDP-choline attenuated the endotoxin-induced increase in pCO_2 (Fig. 5D.) and decrease in pH (Fig. 5F.). Statistical analysis with two-way repeated measures ANOVA revealed a significant effect for choline [$F(1.63) = 11.21$; $P<0.001$] and a dose-time interaction [$F(1.63) = 28.08$; $P<0.001$], as well as a significant effect for CDP-choline [$F(1.70) = 14.28$; $P<0.001$] and a dose-time interaction [$F(1.58) = 5.00$; $P<0.001$] on

endotoxin-induced alterations in pO2. In addition, two-way repeated measures ANOVA also revealed a significant effect for choline [F (1.63) = 36.37; P <0.001] and a dose-time interaction [F (7.63) = 7.28; P <0.001], as well as a significant effect for CDP-choline [F (7.58) = 24.41; P <0.001] and a dose-time interaction [F (7.58) = 6.60; P <0.001] on endotoxin-induced alterations in pCO2.

The calculated parameters from blood gas analyses were summarized in Table 3. Saline or CDP-choline treatment failed to alter any of these calculated parameters (data not shown). Choline also failed to alter base excess, extracellular base excess, O_2 saturation and total CO_2 concentration in plasma ($CtCO_2$), but it decreased slightly HCO_3 standard from 19.9 ± 0.3 mmol/L (P<0.05) to 18.5 ± 0.2 mmol/L, and HCO_3 active from 19.1 ± 0.3 mmol/L to 17.7 ± 0.1 mmol/L (P<0.05) 30 min after the treatment.

HCO_3 standard and HCO_3 active decreased significantly for 4 h after endotoxin (Table 3). These declines were not observed in dogs pre-treated either with choline or CDP-choline (Table 3). Base excess and extracellular base excess values were increased from -3.8 ± 0.6 mmol/L to 9.5 ± 0.7 mmol/L and from -4.2±0.7 to -11.4±0.8 mmol/L 1 h after endotoxin, respectively, and these values gradually returned to their baseline values within 24-48 h after endotoxin. Pre-treatment with either choline or CDP-choline blocked the endotoxin-induced changes in base excess and extracellular base excess values (Table 3). The declines induced by endotoxin in O_2 saturation at 2 h, and $CtCO_2$ at 2-4 h were both blocked by treatment with either choline or CDP-choline (Table 3).

1.4.Effects of choline or CDP-choline on serum electrolytes in control and endotoxemic dogs

Endotoxemia was associated with elevations in serum electrolytes (Fig. 6.) for 1-24 h. Choline or CDP-choline treatment failed to affect serum electrolytes in control dogs (data not shown) but blocked the endotoxin-induced elevations in serum levels of sodium (Fig.

6A.), potassium (Fig. 6B.), calcium (Fig. 6C.), chloride (Fig. 6D.), phosphorus (Fig. 6E.) and magnesium (Fig. 6F.).

1.5.Effects of choline or CDP-choline on plasma TNF-a, HMGB1 protein, catecholamines and NO (nitrate/nitrite) in control and endotoxemic dogs

Choline or CDP-choline failed to alter TNF-α, HMGB1 protein and nitric oxide (NO) levels in control dogs but suppressed the increases in these plasma parameters in response to endotoxin in dogs (Table 4).

Choline or CDP-choline caused a significant increase in plasma catecholamine levels in control dogs (Table 4), and enhanced endotoxin-induced elevation in plasma catecholamine levels at 2 h after the treatment (Table 4).

2. Discussion

These data show that choline or CDP-choline blocks or attenuates endotoxin-induced depression in cardiovascular and respiratory functions. Endotoxemia associates with long-lasting hypotension, tachycardia, changes in cardiac dimensions and functions, elevation in cardiac injury markers, increase in respiratory rate, decrease in pO2, increase in pCO2 and increases in serum electrolytes, NO levels, TNF-α, and HMGB1. In this model of canine endotoxemia, choline or CDP-choline attenuates or prevents these endotoxin-induced alterations in cardiovascular and respiratory functions.

These data extend our previous knowledge by showing that choline or CDP-choline has the ability to prevent and/or attenuate body's responses to bacterial endotoxin (Ilcol et al, 2005 and 2009; Yilmaz et al, 2006, 2010 and 2016; Tvarijonaviciute et al, 2012; Kocaturk et al, 2016; Eralp Inan et al, 2019). As expected (Evans et al, 1984; Ntanson et al, 1984; Forfia et al, 1998; Batmaz et al, 2003), endotoxin produced immediate and sustained severe hypotension (Fig. 1B, D, and F) which was attenuated greatly by choline or restored totally by CDP-choline at all time points during 48 hours of observation period in the course of

endotoxin shock (Fig. 1). The observed attenuation and/or restoration in endotoxin-induced hypotension could have resulted, in part, from the pressor action of choline and CDP-choline at first 0.5-2 h. This view is further supported by previous data showing that choline treatment increases (Ulus et al, 1995; Srimal et al, 1969) and restores blood pressure (Arslan et al, 1991; Savci and Ulus, 1996, 1997 and 1998; Ulus et al, 1995; Gürün et al, 1997 and 2003) in various experimental shock models [i.e., endotoxin shock (Savci and Ulus, 1997), hemorrhagic shock (Ulus et al, 1995; Gurun et al, 2003) and spinal shock (Savci and Ulus, 1998)]. Along the similar lines, CDP-choline also increases and restores blood pressure in hemorrhagic shock (Cansev et al, 2007; Savci et al, 2002 and 2003) and septic shock (Sevim et al, 2017), respectively. Since the pressor action of choline or CDP-choline in control dogs was short-lasting and blood pressure returned to baseline values within few hours (i.e., at 1h or 2 h for choline or CDP-Choline, respectively), the observed attenuation and/or restoration of endotoxin-induced hypotension at 4-48 h by choline and/or CDP-choline likely involves additional mechanism(s) [i.e., attenuation in NO, TNF-α, and HMGB1 protein responses to endotoxin, and improvement in CO (see below)].

In accordance with previous studies (Evans et al, 1984; Natanson et al, 1084; Forfia et al, 1998; Batmaz et al, 2005), endotoxemia resulted in immediate and sustained elevations in heart rate (Fig. 2.), reductions in CO (Fig. 3.) and mitral E/A ratio, and increase in the LV Tei index (echocardiographic index of combined systolic and diastolic function) accompanied with significant changes in myocardial performances, LV functions, and in LV geometric and volumetric parameters (Table 1) in dogs. The observed functional and structural cardiac responses to endotoxin in dogs receiving choline or CDP-choline were much smaller in magnitude and shorter in duration than the observed values in control dogs. Absence of significant changes in all of the measured parameters by CDP-choline in control dogs rule out the possibility that the partial and/or total restorations in

hemodynamic parameters reported in the endotoxin-treated dogs were due to effects of CDP-choline in an opposite direction on the measured cardiac parameters. Except for the heart rate and CO, same conclusion can be drawn for choline for the observed attenuation and or restoration in measured cardiac parameters in endotoxin-treated dogs. The observed decrease in heart rate by choline in both control and endotoxin treated dogs was expected (Sevim et al, 2017), and likely resulted from its direct [i.e., as a muscarinic receptor agonist (Ulus et al, 1988)] and/or indirect [i.e., increasing vagal activity (tone) by stimulating acetylcholine synthesis (Meyer and Baker, 1986; Ilcol et al, 2003)] muscarinic actions on the heart, at the sinus node. Since heart rate is an important determinant of CO, the observed small and short-lasting decrease in CO by choline in control dogs could have resulted from the decrease in heart rate by choline. Despite the increase in heart rate, the CO decreased to the 1/2-1/3 of the initial value in response to endotoxin (Fig. 3A.). The ability of choline or CDP-choline to improve the CO in dogs with endotoxemia apparently resulted mainly, if not solely, from improvement in blood pressure (Fig. 1.) and LV end-diastolic and end-systolic volumes (Table 1) by these two drugs. In a previous study, we showed that choline decreased circulating levels of the cardiac injury marker, CK-MB, in dogs during endotoxemia (Ilcol et al, 2005). In the present study, we confirm and extend this early observation by demonstrating that choline or CDP-choline attenuates, to a great extent, the increases in levels of CK-MB, HBDH, myoglobin, and cTnI, the most specific cardiac injury marker, in response to endotoxin. Taken together, it is reasonable to assume that the observed improvement in cardiac performance and ventricular functions by choline or CDP-choline in dogs with endotoxemia, is a consequence of prevention of myocardial injury in dogs treated either with choline or CDP-choline.

In this study, multifocal atrial tachycardia (MAT) that was observed in endotoxemic dogs may have resulted from increased circulating catecholamine levels for 24 hr after

endotoxin treatment (Table-4). Activation of sympathetic transmission by the enhancement in circulating catecholamines is known to induce arrhythmias, MAT, reentrant tachycardia, atrial fibrillation and wandering atrial pacemaker. The mechanism is thought to be due to sinus node and atrial tissue sensitivity to α- and β-adrenergic stimuli as well as to decreased cardio-vagal reflex. The mechanisms involve autonomic transmitters, changes in impulse formation, conduction and refractoriness (Aburawi et al. 2013).

The observed immediate and sustained increase in respiratory rate (Fig. 4.) and in pCO_2 (Fig. 5.), profound reduction in pO_2 and pH, and adverse changes in measured and calculated blood gases parameters (Table 5) and serum electrolytes (Table 5) indicate severe respiratory dysfunctions as well as metabolic disturbances. In accordance with the observed improvement in cardiovascular function, choline or CDP-choline also improved respiratory functions and electrolyte disturbances. CDP-choline restored pO2 totally and attenuated the changes in respiratory rate, pCO_2, pH and serum electrolytes in response to endotoxin (Fig. 4. and 5.). Choline also improved pO2, pCO_2 and pH (Fig. 5.) and decreased respiration rate in dogs receiving endotoxin, all of the measured blood gas parameters (Table 5) and serum electrolytes (Table 6). On the other hand, choline caused immediate and profound respiratory distress associated with an increase in respiratory rate (Fig. 4A.), and decrease in pCO_2 and pH (Fig. 5C., E.) in control dogs. The respiratory distress and increase in respiratory rate could be explained by activation of chemoreceptors/chemosensors (Sevim et al, 2017) and/or muscarinic receptors at the respiratory track by high concentration of choline chloride during rapid intravenous infusion. In our previous study (Yilmaz et al, 2010) we demonstrated that choline concentration in circulation reaches sufficiently high levels (i.e.>500 μM) to induce bronchoconstriction by activating muscarinic cholinergic receptors. It is also known that the cholinergic system is involved in neural control of respiration (Shao and Feldman,

2009), and enhancement in cholinergic nicotinic neurotransmission increases respiratory rate (Shao and Feldman, 2009). Therefore, the observed increase in respiratory rate could have also resulted, in part, from enhanced nicotinic cholinergic neurotransmission within the brain stem respiratory network (Shao and Feldman, 2009). The observed decrease in pCO_2 and pH is likely the result of increased respiratory rate. It is interesting to note that while the effects of choline alone on respiratory rate, pCO_2 and pH were in the same direction with endotoxin, choline was found to attenuate these parameters in dogs receiving endotoxin. Table 4 shows that plasma catecholamine concentrations are much greater in dogs receiving endotoxin plus choline than those in endotoxin- or choline-treated dogs. Thus, one possible explanation is that the greater levels of circulating catecholamines observed with endotoxin plus choline could have relived bronchoconstriction to some degree by activating α2-adrenoreceptors at the respiratory tract.

The observed significant increases in levels of circulating TNF-α, catecholamine and nitrite/nitrate in dogs in response to endotoxin are consistent with our previous studies (Yilmaz et al, 2008). In the present study, we also observed significant increases in circulating levels of HMGB1 protein. As expected (Rosas-Ballina and TRACEY, 2019; Parrish et al, 2008), the observed increase in NO and HMGB1 were much greater at 24 h than the values observed at 2 h (Table 4). It is known that increases in TNF-α, HMGB1 protein and NO are involved in the dysregulated body's responses to endotoxin at different time points. In the present study, we observed that large increases in TNF-α, HMGB1 and NO during endotoxemia were suppressed by treatment with choline or CDP-choline (Table 4). These findings suggest that beneficial actions of choline and CDP-choline on cardio-vascular and respiratory dysfunction may involve, at least in part, suppression of TNF-α, HMGB-1 protein and NO responses to endotoxin. Contrary to the attenuation of TNF-α, HMGB1 protein and NO, choline or CDP-choline enhanced plasma catecholamine

response to endotoxin (Table 4). Choline is a precursor of the neurotransmitter acetylcholine which stimulates acetylcholine synthesis and augments cholinergic neurotransmission in the sympatho-adrenal system (Ulus et al, 1977), and choline treatment enhances the response of the sympatho-adrenal system to activation (Ulus et al, 1977; Ulus et al, 1978; Ulus et al, 1995; Cansev et al, 2008). Thus, the observed increase in plasma catecholamine response to endotoxin by choline or CDP-choline is in good accordance with these previous studies (Ulus et al, 1977; Ulus et al, 1978; Ulus et al, 1995; Cansev et al, 2008), and may be involved in improvement of hypotension by these drugs (see above).

The present study provides the first systematic data demonstrating the effects of choline or CDP-choline on cardiovascular and respiratory system in dogs. Although the doses of choline chloride (20 mg/kg) and CDP-choline disodium (70 mg/kg) provides the same amount of free choline, we observed some important differences in the observed effects of choline chloride and CDP-choline disodium in the present study. Choline chloride increased blood pressure (Fig. 1A.), decreased heart rate (Fig. 2A.) and increased respiratory rate (Fig. 4A.). Infusion of CDP-choline also increased blood pressure (Fig. 1A.), but failed to alter the heart rate (Figure 1A) and respiratory rate (Fig. 4A.). In addition to the respiratory distress side effect of choline treatment, discussed above, choline administration also produced a number of muscarinic cholinergic symptoms, including diarrhea, vomiting and salivation. These side effects were observed in 60 % of dogs receiving choline treatment. Not all side effects occurred in a dog at the same time and, when occurred, each of them was well-tolerated by the dogs and none of them necessitated termination of the study. Hence, although these side effects may seem a potential contraindication for using choline in endotoxic shock, choline treatment in this

disorder would be a matter of preference due to the benefit that choline provided against its side effects.

On the other hand, CDP-choline did not produce the above-mentioned side effects and was more effective than the equivalent dose of choline for restoring blood pressure and improving cardiac and respiratory functions (see results), as noted previously (Yilmaz et al, 2010). In the present study, we did not measure free choline concentrations, but in our previous studies we noted that plasma concentrations of free choline attained 500-1000 μM or 200-300 μM following intravenous infusion of 20 mg/kg of choline chloride (Yilmaz et al, 2010) or 70 mg/kg of CDP-choline disodium (Yilmaz et al, 2006), respectively. Choline increases cholinergic neurotransmission by acting as a precursor for the neurotransmitter acetylcholine (Ulus and Wurtman, 1976; Ulus et al, 1977; Meyer and Baker, 1986; Johnson et al, 1992, Buyukuysal et al, 1995; Ulus et al, 2006), but it also acts as a direct agonist for both muscarinic and nicotinic cholinergic receptors at >500 μM concentration (Ulus et al, 1988). As discussed above, the observed decrease in heart rate or the respiratory distress after infusion of choline likely resulted from the activation of muscarinic receptors in cardiac pacemaker cells or those at respiratory tract smooth muscle (Ulus et al, 1988), respectively. The increase in respiratory rate could also have resulted from direct nicotinic action of choline at the chemoreceptors/chemosensor (Ulus et al, 1988; Shao and Feldman, 2009).

Exogenously-administered CDP-choline is metabolized to cytidine monophosphate and phosphocholine which subsequently are dephosphorylated to cytidine and choline, respectively (Wurtman et al, 2000; Cansev et al, 2007). Circulating concentrations of CDP-choline itself and of its metabolites cytidine monophosphate (CMP), phosphocholine, choline and cytidine are increased in a dose-dependent manner following CDP-choline treatment (Cansev et al, 2007). CMP, phosphocholine or cytidine affects blood pressure

and heart rate in the opposite direction compared to choline (Cansev et al, 2007). Thus, the observed differences between choline and CDP-choline could be explained by the difference in attainable free choline concentrations and/or actions of other CDP-choline metabolites (i.e., CMP, phosphocholine and cytidine).

3. Conclusion

In conclusion, the findings reported here show that choline, as choline chloride or CDP-choline, restores blood pressure and improves cardiac and respiratory dysfunction in dogs with experimentally-induced endotoxemia in this study. The present findings in dogs may be directly applicable to clinical practice in veterinary medicine with regard to the treatment of septic/endotoxic shock. In addition, the findings of this study also point out the potential use of choline, particularly as CDP-choline (less toxic, more effective with fewer side effects than equivalent dose of choline chloride) in humans with endotoxic shock, as well. At the time of the preparation of this manuscript, the pandemic of Covid-19 has occurred, and apparently the lung injury and development of the respiratory/cardiovascular dysfunctions increased mortality, as in the case for septic/endotoxic shock. Although this should be taken with caution, based on the results of our studies, it could be postulated that choline and/or CDP-choline treatment could confer significant benefit in patients with Covid-19 at risk due to dysfunctions of respiratory and cardiovascular systems.

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Declaration of Competing Interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Funding

This study was supported by The Turkish Scientific and Technical Research Council (TUBITAK, Project no: 109O660) and by The Turkish Academy of Science (Prof. Dr. Ismail H. Ulus). This study is a part of Ph.D. thesis of Meric Kocaturk.

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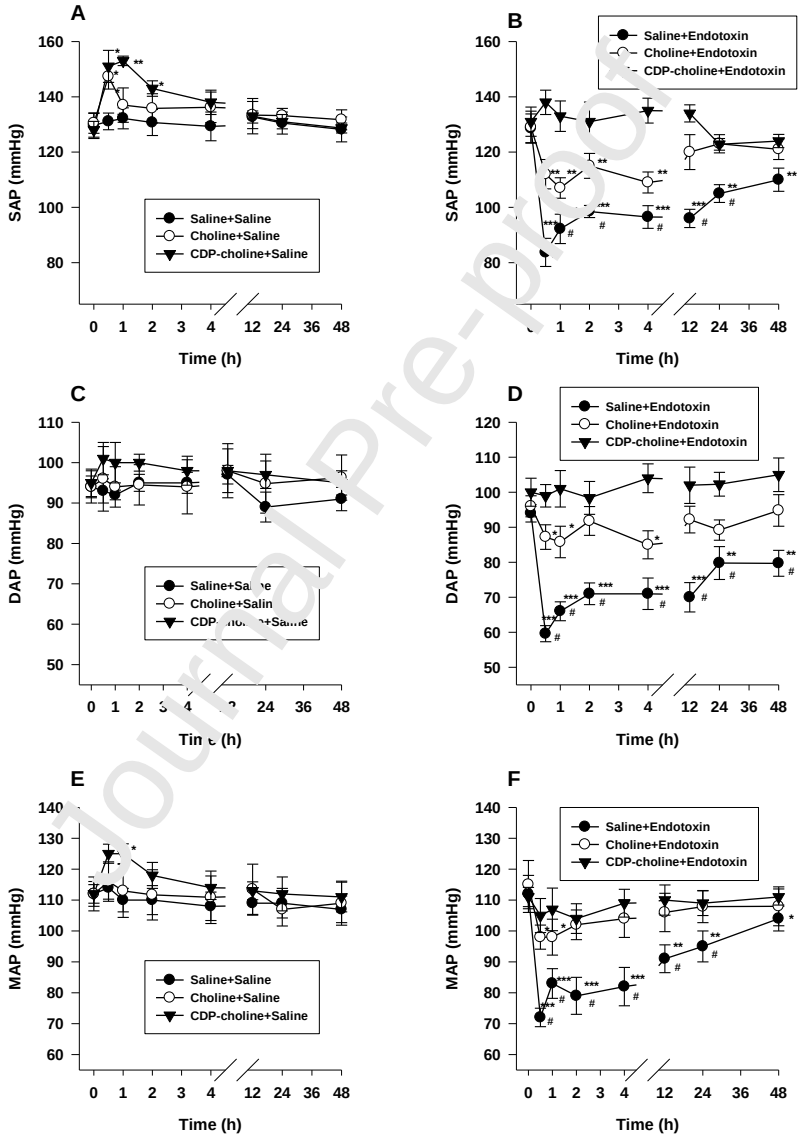
Fig. 1. Effects of choline or CDP-choline on endotoxin-induced changes in systolic, diastolic and mean arterial pressure in dogs.



Dogs were injected i.v. with saline or saline containing endotoxin (0.1 mg/kg). Five minutes after the first i.v. treatment, dogs received saline or saline containing choline (20

mg/kg) or CDP-choline (70 mg/kg). SAP, DAP and MAP were measured at given time points. Each point represents mean ± SEM of 4-6 determinations. *P<0.05, **P<0.01 and ***P<0.001 when compared with the baseline value (0 h). #Different (P<0.05-0.001) from the respective values of "Choline+Endotoxin" and/or "CDP-choline+Endotoxin" groups. SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure.

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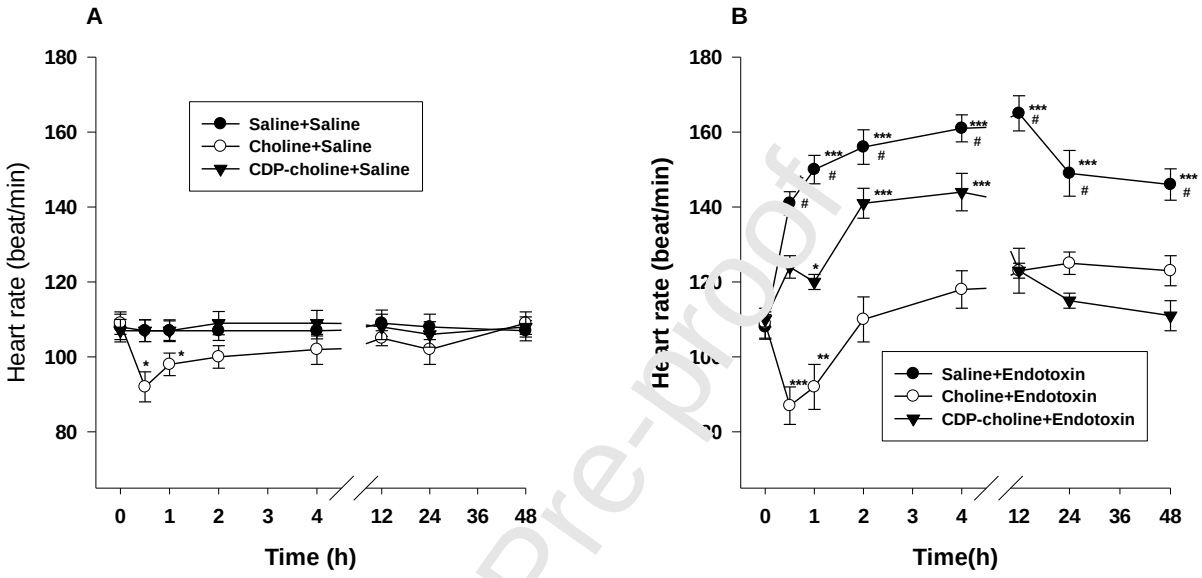
Fig. 2. Effects of choline or CDP-choline on endotoxin-induced changes in heart rate in dogs.



Dogs were injected i.v. with saline or saline containing endotoxin (0.1 mg/kg). Five minutes after the first i.v. treatment, dogs received saline or saline containing choline (20 mg/kg) or CDP-choline (70 mg/kg). Heart rate was measured at given time points. Each point represents mean + SEM of 4-6 determinations. *P<0.05, **P<0.01 and ***P<0.001 when compared with the baseline value (0 h). #Different (P<0.05-0.001) from the respective values of "Choline+Endotoxin" and/or "CDP-choline+Endotoxin" groups

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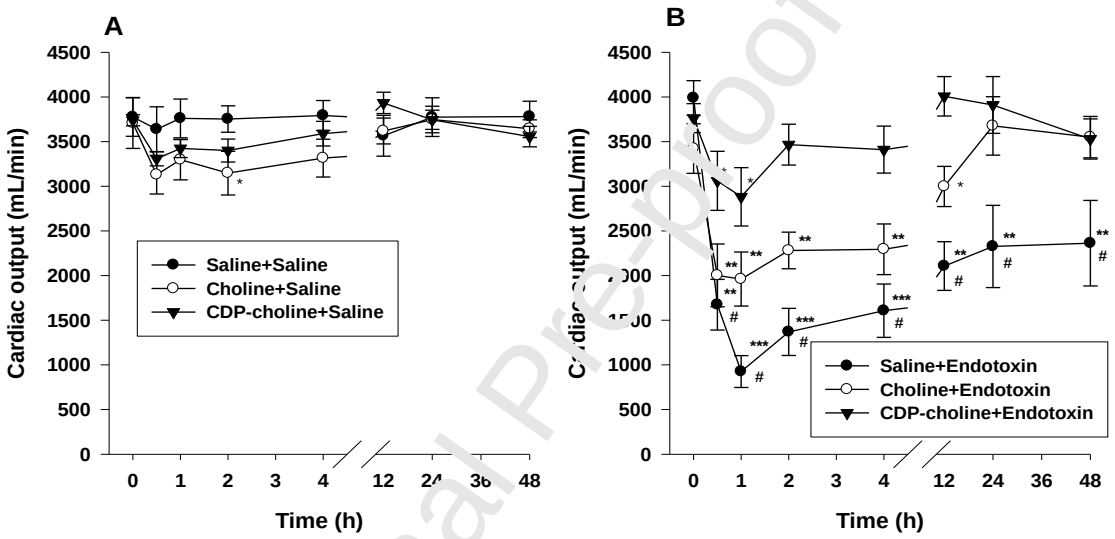
Fig. 3. Effects of choline or CDP-choline on endotoxin-induced changes in cardiac output in dogs.



Dogs were injected i.v. with saline or saline containing endotoxin (0.1 mg/kg). Five minutes after the first i.v. treatment, dogs received saline or saline containing choline (20 mg/kg) or CDP-choline (70 mg/kg). Cardiac output was estimated from stroke volume and heart rate calculated by echocardiography and electrocardiography, respectively. Each point represents mean ± SEM of 4-6 determinations. *P<0.05, **P<0.01 and ***P<0.001 when compared with the baseline value (0 h). #Different (P<0.05-0.001) from the respective values of "Choline+Endotoxin" and/or "CDP-choline+Endotoxin" groups.

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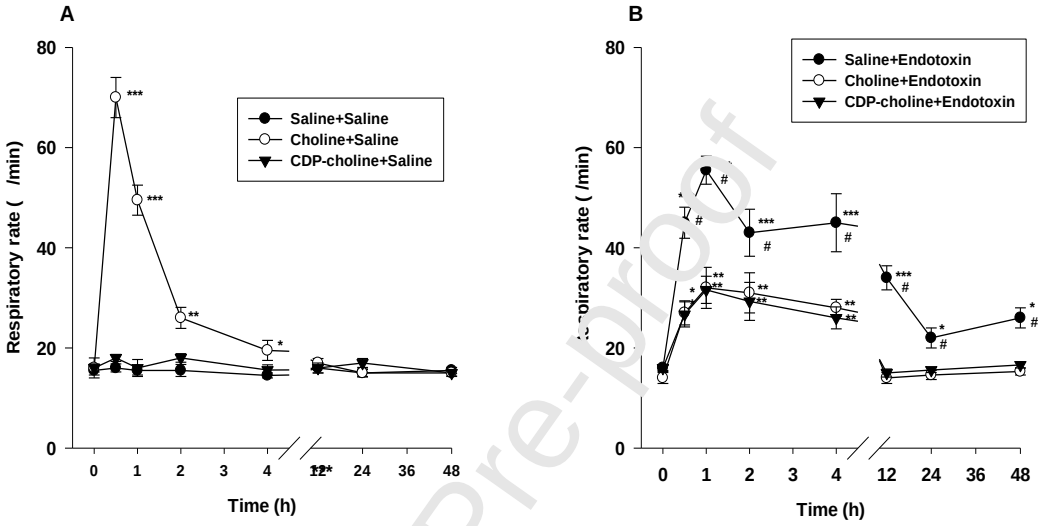
Fig. 4. Effects of choline or CDP-choline on endotoxin-induced changes in respiratory rate in dogs.



Dogs were injected i.v. with saline or saline containing endotoxin (0.1 mg/kg). Five minutes after the first i.v. treatment, dogs received saline or saline containing choline (20 mg/kg) or CDP-choline (20 mg/kg). Respiratory rate was measured at given time points. Each point represents mean ± SEM of 4-6 determinations. *P<0.05, **P<0.01 and ***P<0.001 when compared with the baseline value (0 h). #Different (P<0.05-0.001) from the respective values of "Choline+Endotoxin" and/or "CDP-choline+Endotoxin" groups.

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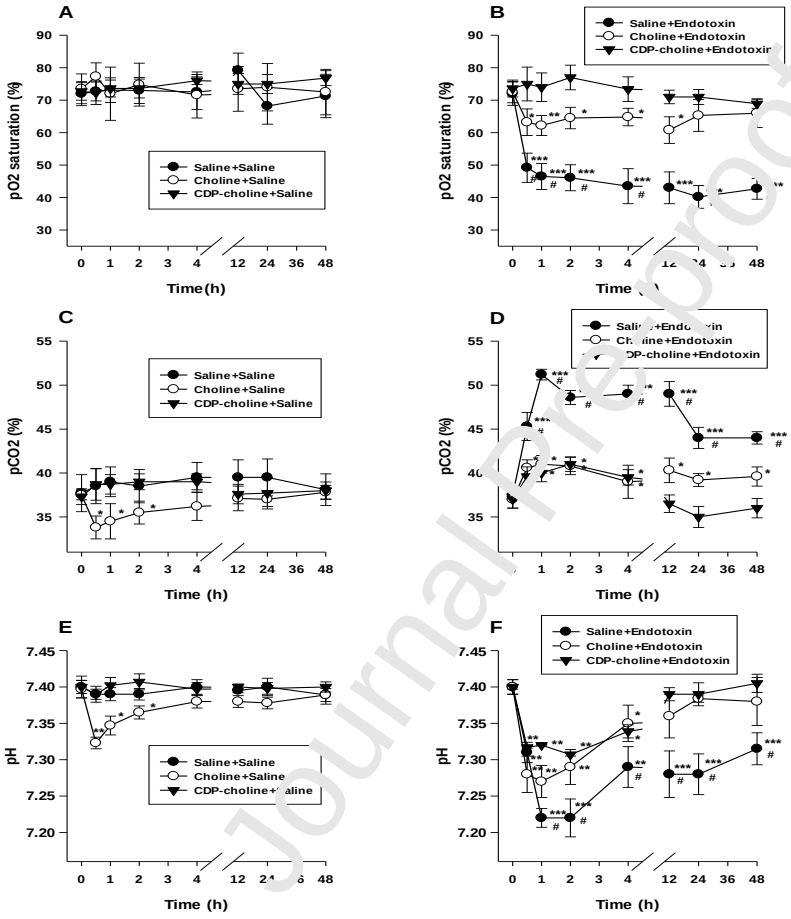
Fig. 5. Effects of choline or CDP-choline on endotoxin-induced changes in pO_2, pCO_2, and pH in dogs.



Dogs were injected i.v. with saline or saline containing endotoxin (0.1 mg/kg). Five minutes after the first i.v. treatment, dogs received saline or saline containing choline (20 mg/kg) or CDP-choline (70 mg/kg). Blood pO2, pCO2 and pH were measured in blood samples obtained at given time points. Each point represents mean ± SEM of 4-6 determinations. *P<0.05, **P<0.01 and ***P<0.001 when compared with the baseline

value (0 h). [#]Different (P<0.05-0.001) from the respective values of “Choline+endotoxin” and/or “CDP-choline+endotoxin” groups.

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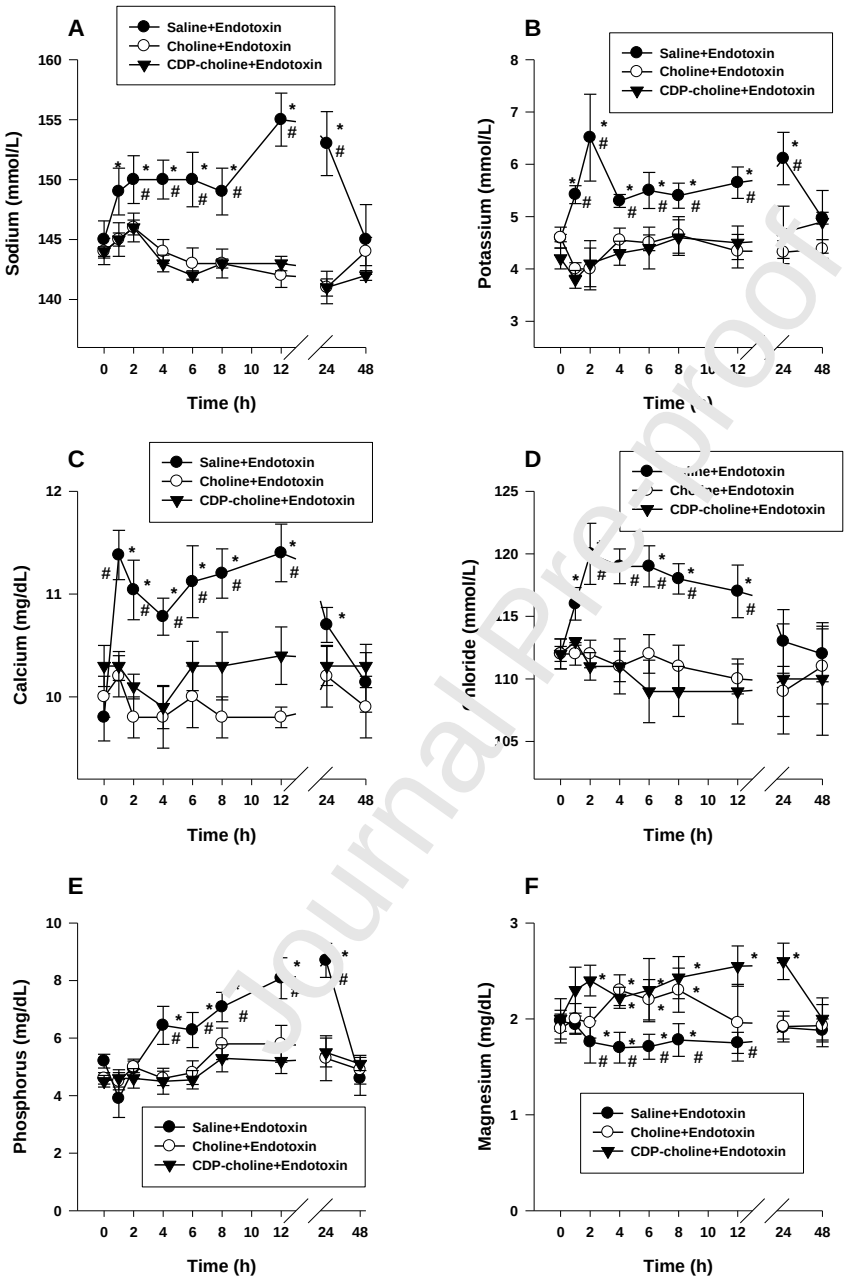
Fig. 6. Effects of choline or CDP-choline on endotoxin-induced changes in serum electrolytes in dogs.



Dogs were injected i.v. with saline or saline containing endotoxin (0.1 mg/kg). Five minutes after the first i.v. treatment, dogs received saline or saline containing choline (20 mg/kg) or CDP-choline (70 mg/kg). Serum electrolytes were measured in blood samples

obtained at given time points. Each point represents mean ± SEM of 4-6 determinations. *P<0.05, **P<0.01 and ***P<0.001 when compared with the baseline value (0 h). #Different (*P*<0.05-0.001) from the respective values of "Choline+Endotoxin" and/or "CDP-choline+Endotoxin" groups.

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Table 1. Effects of choline or CDP-choline on endotoxin-induced changes in left ventricular geometry and volume parameters determined by echocardiographic analysis.

Parameters /Treatments	Baseline	Time After Treatment 2 h	4 h	12 h	24 h	48 h
LVSD (cm)						
E+S	0.96±0.05	1.97±0.07*	2.00±0.05*	2.01±0.08*	2.02±0.05*	2.06±0.04*
E+Ch	0.90±0.04	0.93±0.05	0.97±0.04	0.96±0.05	0.95±0.06	1.00±0.04
E+CDP-Ch	0.91±0.04	0.93±0.05	0.98±0.05	1.04±0.05	0.98±0.04	0.96±0.07
LVDD (cm)						
E+S	3.80±0.18	2.72±0.18*	2.98±0.26*	3.70±0.21	3.75±0.17	3.75±0.19
E+Ch	4.15±0.18	3.53±0.05	3.76±0.00	3.83±0.14	4.05±0.14	4.20±0.24
E+CDP-Ch	4.11±0.16	3.50±0.17	3.78±0.14	3.90±0.16	4.02±0.12	4.30±0.28
IVSSD (cm)						
E+S	1.28±0.06	2.30±0.08*	2.08±0.03*	2.20±0.04*	2.13±0.04*	2.10±0.03*
E+Ch	1.35±0.05	1.30±0.00	1.32±0.05	1.30±0.06	1.33±0.09	1.37±0.08
E+CDP-Ch	1.35±0.04	1.30±0.05	1.28±0.05	1.32±0.07	1.30±0.05	1.28±0.06
IVSDD (cm)						
E+S	0.96±0.05	1.95±0.04*	2.00±0.05*	2.02±0.08*	2.02±0.05*	2.05±0.05*
E+Ch	0.89±0.04	0.93±0.05	0.97±0.04	0.98±0.06	0.95±0.06	1.00±0.06
E+CDP-Ch	0.91±0.04	1.04±0.05	0.96±0.05	0.98±0.04	0.96±0.07	0.92±0.06
LVPWSD (cm)						
E+S	1.45±0.05	2.20±0.10*	2.08±0.08*	2.05±0.11*	2.02±0.12*	2.03±0.15*
E+Ch	1.42±0.03	1.48±0.05	1.53±0.02	1.42±0.04	1.48±0.05	1.43±0.06
E+CDP-Ch	1.50±0.04	1.57±0.02	1.47±0.03	1.45±0.03	1.47±0.06	1.47±0.02
LVPWDD (cm)						
E+S	1.32±0.05	2.05±0.09*	2.00±0.06*	1.92±0.12*	1.87±0.13*	1.87±0.14*
E+Ch	1.25±0.04	1.37±0.06	1.30±0.03	1.28±0.05	1.30±0.06	1.23±0.06
E+CDP-Ch	1.21±0.03	1.30±0.07	1.26±0.02	1.22±0.02	1.22±0.04	1.16±0.02
LVVS (ml)						
E+S	8.9±0.9	4.0±0.5*	4.1±0.6*	5.9±0.8*	7.1±0.6	8.2±0.4
E+Ch	9.2±0.6	7.2±0.5	7.6±0.8	8.0±0.8	8.5±0.7	9.4±0.4
E+CDP-Ch	9.2±0.7	8.7±0.8	8.7±0.9	9.5±0.9	9.1±1.2	9.1±0.8
LVVD (ml)						
E+S	34.2±3.6	15.7±2.2*	16.1±2.3*	27.5±2.6	29.7±2.7	30.7±3.4
E+Ch	35.0±2.2	31.0±2.7	32.8±2.4	33.0±2.3	32.5±2.0	34.7±1.1
E+CDP-Ch	37.1±2.2	37.3±1.8	37.0±1.9	37.1±1.9	37.6±2.3	36.3±2.2

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Dogs were injected i.v. with endotoxin (0.1 mg/kg). Five minutes after the first i.v. treatment, dogs received saline or saline containing choline (20 mg/kg) or CDP-choline (70 mg/Kg). The left ventricular geometry and volume parameters determined by echocardiographic analysis at given time points. Each point represents mean ± SEM of 4-6 determinations. *Significantly ($P<0.05$-0.001) different from the baseline value (by one-way repeated measures ANOVA), and the respective values of "E+Ch" and/or "E+CDP-Ch" groups (by two-way repeated measures ANOVA). E=Endotoxin, S= Saline, Ch= Choline, CDP-Ch = CDP-Choline.

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Table 2. Effects of choline or CDP-choline on endotoxin-induced changes in cardiac injury markers.

Parameters /Treatments	Time After Treatment				
	2 h	4 h	12 h	24 h	48 h
CK-MB (IU/L)					
E+S	1492±161*	1878±189*	1356±167*	796±72*	490±70*
E+Ch	612±94	869±103	585±102	427±74	252±72
E+CDP-Ch	405±94	565±44	654±126	486±92	232±41
HBDH (IU/L)					
E+S	ND	1305±140*	ND	1207±274*	ND
E+Ch	ND	360±30	ND	364±61	ND
E+CDP-Ch					
Myoglobin (mg/dL)					
E+S	ND	22.8±2.9*	ND	12.0±1.2*	ND
E+Ch	ND	10.5±0.5	ND	4.8±0.6	ND
E+CDP-Ch	ND	3.6±0.4	ND	2.1±0.2	ND
cTnI (ng/mL)					
E+S	ND	8.3±0.5*	ND	12.7±0.9*	ND
E+Ch	ND	1.7±0.3	ND	3.9±0.7	ND
E+CDP-Ch	ND	0.6±0.1	ND	0.8±0.3	ND

Dogs were treated as described at the method section and at the legend of Table 1. Cardiac injury markers were assayed in blood samples collected at the given time points. Each point represents mean ± SEM of 4-6 determinations. *Significantly ($P<0.05$-0.001) different from the baseline value (by one-way repeated measures ANOVA), and the respective values of "E+Ch" and/or "E+CDP-Ch" groups (by two-way repeated measures ANOVA). E=Endotoxin, S= Saline, Ch= Choline, CDP-Ch = CDP-Choline.

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Table 3. Effects of choline or CDP-choline on endotoxin-induced changes in blood gas analysis parameters.

Parameters /Treatments	Baseline	Time After Treatment 2 h	4 h	12 h	24 h	48 h
HCO_3 standard (mmol/L)						
E+S	22.5±0.6	17.0±0.2*,#	18.3±0.6*	19.9±0.9*	21.0±0.8	21.3±0.6
E+Ch	21.7±0.7	19.9±0.4	20.7±0.4	21.7±0.2	21.6±0.8	22.3±0.3
E+CDP-Ch	22.7±0.6	23.1±0.4	22.9±0.2	23.4±0.5	22.7±0.5	22.6±0.6
HCO_3 active (mmol/L)						
E+S	21.9±0.6	13.5±0.8*,#	15.4±0.6*,#	20.4±0.4	21.2±0.7	21.8±0.2
E+Ch	22.3±0.6	22.0±0.9	22.7±0.2	21.5±0.3	22.8±0.8	22.7±0.3
E+CDP-Ch	22.0±0.6	22.2±0.8	22.6±0.7	23.5±0.6	23.1±0.6	22.3±0.6
Base excess (mmol/L)						
E+S	-3.8±0.6	-9.0±0.4*,#	-8.3±0.9*,#	-5.9±0.6*	-6.0±0.3*,#	-5.8±0.5*#
E+Ch	-3.6±0.2	-5.6±0.2*	-4.7±0.2	-4.9±0.2*	-4.1±0.3	-3.7±0.1
E+CDP-Ch	-3.9±0.4	-5.5±0.2	-4.7±0.3	-4.5±0.4	-4.4±0.3	-4.2±0.4
Extracellular Fluid Base excess (mmol/L)						
E+S	-4.2±0.7	-10.1±0.7*,#	-10.3±0.8*,#	-5.7±0.9	-5.6±0.8	-5.4±0.7
E+Ch	-3.6±0.2	-5.6±0.2*	-6.8±0.4*	-5.4±0.1*	-4.3±0.2	-4.7±0.4
E+CDP-Ch	-3.9±0.2	-4.1±0.2	-3.7±0.2	-3.3±0.2	-3.6±0.2	-3.2±0.5
O_2 Saturation (%)						
E+S	78.7±3.1	52.7±4.6*,#	78.7±1.0	80.2±1.2	74.2±2.8	71.4±2.8
E+Ch	81.0±3.4	81.0±2.3	86.5±1.2	80.0±2.3	83.5±2.5	84.0±1.0
E+CDP-Ch	79.0±1.7	78.0±1.7	84.6±3.8	85.5±2.5	86.0±1.6	85.5±2.5
$CtCO_2$						
E+S	20.9±0.8	16.3±1.0*,#	16.8±1.5*,#	21.8±0.4	23.3±0.6	21.6±0.6
E+Ch	22.8±0.6	22.9±0.4	23.8±0.9	23.1±0.7	23.3±0.8	23.6±0.7
E+CDP-Ch	23.6±1.1	23.2±0.9	22.7±0.5	23.1±0.6	24.7±0.7	24.6±0.7

Dogs were treated as described at the legend of Table 1. Each point represents mean ± SEM of 4-6 determinations. *Significantly ($P<0.05$-0.001) different from the baseline value (by one-way repeated measures ANOVA), and the respective values of "E+Ch"

and/or “E+CDP-Ch” groups (by two-way repeated measures ANOVA). E=Endotoxin, S= Saline, Ch= Choline, CDP-Ch = CDP-Choline.

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Table 4. Effects of choline or CDP-choline on endotoxin-induced changes in blood TNF-α, HMGB1 protein, catecholamine and NO levels.

Parameters/ Treatments	Time After Treatment	
	2 h	24 h
TNF-α <ng/L		
S+S	33 ± 11	ND
E+S	323 ± 27*,#	ND
S+Ch	27 ± 5	ND
E+Ch	161 ± 13*	ND
S+CDP-Ch	28 ± 9	ND
E+CDP-Ch	115 ± 9*	ND
HMGB1 Protein (ng/L)		
S+S	8 ± 1	7 ± 1
E+S	52 ± 6*,#	93 ± 8*,#
S+Ch	7 ± 1	8 ± 1
E+Ch	17 ± 3*	26 ± 7*
S+CDP-Ch	7 ± 1	7 ±1
E+CDP-Ch	39 ± 4*	41 ± 7*
Catecholamine (ng/L)		
S+S	45 ± 10	38 ± 12
E+S	940 ± 115*,#	375 ± 40*
S+Ch	215 ± 25	ND
E+Ch	1475 ± 140*	ND
S+CDP-Ch	180 ± 25	ND
E+CDP-Ch	1510 ± 150*	ND
NO (nmol/L)		
S+S	1.1 ± 0.5	1.1 ± 0.4
E+S	4.3 ± 0.9*	27.4 ± 3.2*,#
S+Ch	1.5 ± 0.4	1.2 ± 0.5
E+Ch	3.0 ± 0.7	15.4 ± 2.2*
S+CDP-Ch	0.9 ± 0.3	0.9 ±1.0
E+CDP-Ch	2.8 ± 0.9	13.5 ± 1.9*

Dogs were treated as described at the legend of Table 1. TNF-α, HMGB1 protein, catecholamine and NO were assayed in blood samples collected at the given time points. Each point represents mean ± SEM of 4-6 determinations. *Significantly (P<0.05-0.001) different from the value observed in respective the "S+S" group. #Significantly (P<0.05-0.001) different from the value observed in the "Ch+E" and/or "CDP-Ch+E" groups.

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Highlights

- Endotoxin shock associates with severe impairment in cardiovascular and respiratory functions
- Choline or cytidine-5'-diphosphocholine (CDP-choline) provides beneficial effects in experimental endotoxin shock in dogs
- Choline or CDP-choline attenuates or prevents endotoxin-induced alterations in cardiovascular and respiratory functions in dogs with experimental endotoxemia

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