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Thromboelastographic evaluation of the effectiveness of choline or CDP-choline treatment on endotoxin-induced hemostatic alterations in dogs

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ABSTRACT

Sepsis/endotoxemia associates with coagulation abnormalities. We showed previously that exogenous choline treatment reversed the changes in platelet count and function as well as prevented disseminated intravascular coagulation (DIC) in endotoxemic dogs. The aim of this follow-up study was to evaluate the effect of treatment with choline or cytidine-5′-diphosphocholine (CDP-choline), a choline donor, on endotoxin-induced hemostatic alterations using thromboelastography (TEG). Dogs were randomized to six groups and received intravenously (iv) saline, choline (20 mg/kg) or CDP-choline (70 mg/kg) in the control groups, whereas endotoxin (0.1 mg/kg, iv) was used alone or in combination with choline or CDP-choline at the same doses in the treatment groups. TEG variables including R- and K-time (clot formation), maximum amplitude (MA) and α-angle (clot stability), G value (clot elasticity), and EPL, A, and LY30 (fibrinolysis), as well as overall assessment of coagulation (coagulation index - CI), were measured before and at 0.5–48 h after the treatments. TEG parameters did not change significantly in the control groups, except for CI parameter after choline administration. Endotoxemia resulted in increased R-time and A value ($P < 0.05$), decreased K-time ($P < 0.05$), α-angle ($P < 0.001$) and CI values ($P < 0.01$) at different time points. Treatment with either choline or CDP-choline attenuated or prevented completely the alterations in TEG parameters in endotoxemic dogs with CDP-choline being more effective. These results confirm and extend the effectiveness of choline or CDP-choline in endotoxemia by further demonstrating their efficacy in attenuating or preventing the altered viscoelastic properties of blood clot measured by TEG.

1. Introduction

Sepsis is a potentially life-threatening organ dysfunction caused by a dysregulated host response to infection (Rosas-Ballina and Tracey, 2009). Pathologically, systemic inflammation can initiate coagulation activation, leading to organ dysfunction, and ultimately to multiple organ failure and death (Rosas-Ballina and Tracey, 2009; Zhang and Ning, 2021). The inflammasomes are cytosolic multiprotein signaling complexes that control the host response to diverse pathogen-associated molecular patterns (PAMPs) from microorganisms. Lipopolysaccharides (LPS), the main component of the outer membrane of Gram-negative bacteria, is a prototypical PAMPs for studying innate immune response (Zhang and Ning, 2021; Gauthier et al., 2022). Endotoxin triggers dysregulated host responses, tissue injury, organ dysfunction, shock and can cause the activation of coagulation, resulting in primary, secondary, and/or tertiary hemostasis abnormalities (Eralp et al., 2011). The suppressing excessive production of toxic mediators in endotoxin-sensitive cells is one of main target in the treatment of hemostatic abnormalities in septic/endotoxic shock (Zhang and Ning, 2021).

Accumulated evidences indicate that LPS-responsive immune cells functions modulated by the neuronal and the non-neuronal cholinergic systems (Rosas-Ballina and Tracey, 2009) described as the “cholinergic

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anti-inflammatory pathway" (Borovikova et al., 2000) which involves an interaction between the Vagus nerve, Acetylcholine (ACh), the alpha-7 nicotinic acetylcholine receptor and the immune system (Wang et al., 2003; Rosas-Ballina and Tracey, 2009). In good accord, several studies reported beneficial outcomes of activating the cholinergic anti-inflammatory system both in terms of regulating host inflammatory responses and alleviating coagulation abnormalities (McGrath et al., 2004; van Westerloo et al., 2006; Ulloa, 2011; Pinder et al., 2019; Andersson, 2020; Qin et al., 2021; Lv et al., 2021). Studies by our group in dogs (Yilmaz et al., 2005, 2006, 2008, and Yilmaz et al., 2010; Ilcol et al., 2009; Kocaturk et al., 2021), calves (Yilmaz et al., 2016) and rats (Ilcol et al., 2009) have shown that treatment with choline, is a precursor of ACh and known to enhance cholinergic activity (Ulus and Wurtman, 1976; Ulus et al., 1977; Meyer and Baker, 1986; Ulus et al., 2006; Johnson and North, 1992; Buyukuysal et al., 1995), and produces cholinergic effects (Savci et al., 2002; Ozarda Ilcol et al., 2002; Ilcol et al., 2003), reduces endotoxin-induced inflammatory response markers (Parrish et al., 2008; Ilcol et al., 2005) and multi organ injury (Ilcol et al., 2005), restores hypotension and improves myocardial and respiratory functions (Kocaturk et al., 2021), and the decline in circulating platelet (PLT) counts (Yilmaz et al., 2006), improves PLT functions (Yilmaz et al., 2006) and coagulation abnormalities (Yilmaz et al., 2010), prevent disseminated intravascular coagulation (DIC) (Yilmaz et al., 2010), attenuates the elevation in lipids levels (Ilcol et al., 2009), alterations in blood cells counts (Yilmaz et al., 2016) and in some specific serum proteins (i.e, matrix metalloproteinases, matrix metalloproteinase tissue inhibitors and immunoglobulins) concentrations (Kocaturk et al., 2016) 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 to restore endotoxin- induced hypotension (Kocaturk et al., 2021), to improve myocardial and respiratory impairments (Kocaturk et al., 2021), and PLT dysfunctions, coagulation abnormalities (Parrish et al., 2008; Ilcol et al., 2005), and to prevent DIC (Yilmaz et al., 2010) in dogs.

TEG is successfully and broadly used, especially for the assessment of a hypercoagulable state, in human and veterinary medicine (Donahue and Otto, 2005). TEG measurements are based on viscoelastic features of the whole blood clot formation, indicating PLT function, clotting time, clot kinetics, clot strength, clot elasticity, and fibrinolysis (Eralp et al., 2011; Col et al., 2013; Yilmaz et al., 2017). TEG evaluations involve the whole coagulation process, which gives the ability to reflect hypocoagulable and hypercoagulable states in a short period of time (Engelen et al., 2017). In addition, TEG allows to evaluate both cellular and plasma protein contributions along with coagulation factor functions during the clotting process, thereby providing advantages to the traditional coagulation screening methods (Ahmad et al., 2016). Our previous studies showed alterations of the coagulation system by TEG evaluations in dogs with endotoxemia (Eralp et al., 2011), dilated cardiomyopathy (Yilmaz et al., 2017), and ehrlichiosis (Kocaturk et al., 2023). Effects of CDP-choline on TEG parameters was also investigated in one study in trauma hemorrhage model in rats (Cam et al., 2015). Despite reported changes of TEG values in endotoxemic dogs (Eralp et al., 2011) and the findings of choline and CDP-choline on endotoxin-induced coagulation abnormalities in dogs by traditional coagulation and PLT function assays (Parrish et al., 2008; Ilcol et al., 2005; Yilmaz et al., 2017), the effect of these agents on viscoelastic properties of blood from initial clot formation to fibrinolysis remains to be identified. Thus, in this follow-up study, we aimed to investigate the effect of choline or CDP-choline on endotoxin-induced hemostatic alterations using TEG in healthy dogs and dogs with endotoxemia. In this regard, the present study provides additional information on the effects of the above-mentioned compounds using TEG compared to the previous report (Eralp et al., 2011).

2. Material and methods

2.1. Dogs

The study material was collected during a previous study (Kocaturk et al., 2021) in which thirty-six cross-bred male dogs, aged between 1 and 6 years and weighing 15–30 kg were included. Health conditions were evaluated based on physical, hematological and serum biochemistry findings. At least 10 days before the start of the experiment, dogs were hospitalized at the Bursa Uludag University Veterinary Teaching Hospital. A standard pellet diet was provided once a day and water access was *ad libitum*. This study was ethically approved by the Animal Care and Use Committee of Bursa Uludag University, Bursa, Turkey (10.02.2009, 2009–01 / 08).

2.2. Study design

Six groups containing six healthy dogs in each were designed. The groups were determined as: Saline (first group), Choline (second group), CDP-choline (third group), LPS (Endotoxin) (fourth group), LPS + Choline (fifth group) and LPS + CDP-Choline (sixth group), as reported in our previous study (Kocaturk et al., 2021). Dogs in the first three groups received saline initially which was followed 5 min later by saline, choline or CDP-choline, respectively. Dogs in the last three groups received endotoxin (LPS) initially which was followed 5 min later by saline, choline or CDP-choline, respectively. All injections were performed intravenously and dosage details were shown in Table 1.

2.3. Sampling

The study required 48 h of monitoring. Venous blood samples were collected using a 18-gauge nonheparinized catheter from all dogs in each group 6 times; before (0 h - baseline) and 0.5, 1, 4, 24, and 48 h after the treatments from the brachiocephalic veins. Samples for TEG analyses were collected into vacutainer tubes containing 3.18% trisodium citrate anticoagulant. Samples for hematology, serum biochemistry and blood gas analyses were collected into appropriate tubes after the TEG sampling, and their results were reported in a previous study (Kocaturk et al., 2021).

2.4. TEG analysis

Kaolin-activated TEG analyses were performed according to the

Table 1
Details of treatment groups.

Groups	n	Administrations
Saline	6	0.2 mg/kg intravenous (iv) sterile saline injection (0.9% NaCl); 5 min. Later second 0.2 mg/kg/iv sterile saline injection
Choline	6	0.2 mg/kg/iv sterile saline injection (0.9% NaCl); 5 min. Later 0.2 mL/kg/iv sterile saline solution containing 20 mg/kg choline*
CDP-choline	6	0.2 mg/kg/iv sterile saline injection (0.9% NaCl); 5 min. Later 0.2 mL/kg/iv sterile saline solution containing 70 mg/kg CDP-choline*
LPS (Endotoxin)	6	0.2 mL/kg/iv sterile saline solution containing 0.1 mg/kg endotoxin*; 5 min. Later 0.2 mg/kg/iv sterile saline injection (0.9% NaCl)
LPS + Choline	6	0.2 mL/kg/iv sterile saline solution containing 0.1 mg/kg endotoxin; 5 min. Later 0.2 mL/kg/iv sterile saline solution containing 20 mg/kg choline
LPS + CDP-Choline	6	0.2 mL/kg/iv sterile saline solution containing 0.1 mg/kg endotoxin; 5 min. Later 0.2 mL/kg/iv sterile saline solution containing 70 mg/kg CDP-choline

* The doses of choline (20 mg/kg), CDP-choline (70 mg/kg) and endotoxin (0.1 mg/kg) were established from our previous studies in dogs (Yilmaz et al., 2006 and Yilmaz et al., 2010; Ilcol et al., 2005 and 2009).

manufacturer's recommendations using a two channel TEG® 5000 Thromboelastograph® Hemostasis System (TEG System, Haemonetics, USA). Calibration and e-tests (electronic tests) after each system opening were performed by following manufacturer's recommendations, as performed in our previous studies (Eralp et al., 2011; Eralp Inan et al., 2016; Yilmaz et al., 2017). Citrated blood samples were transferred to 1 mL vials containing kaolin and mixed. Then, 320 μL of the kaolin blood sample was added into cups, placed in the TEG channel containing 20 μL $CaCl_2$. Analyses were performed by the same operator and each sample was run within 1 h after sampling.

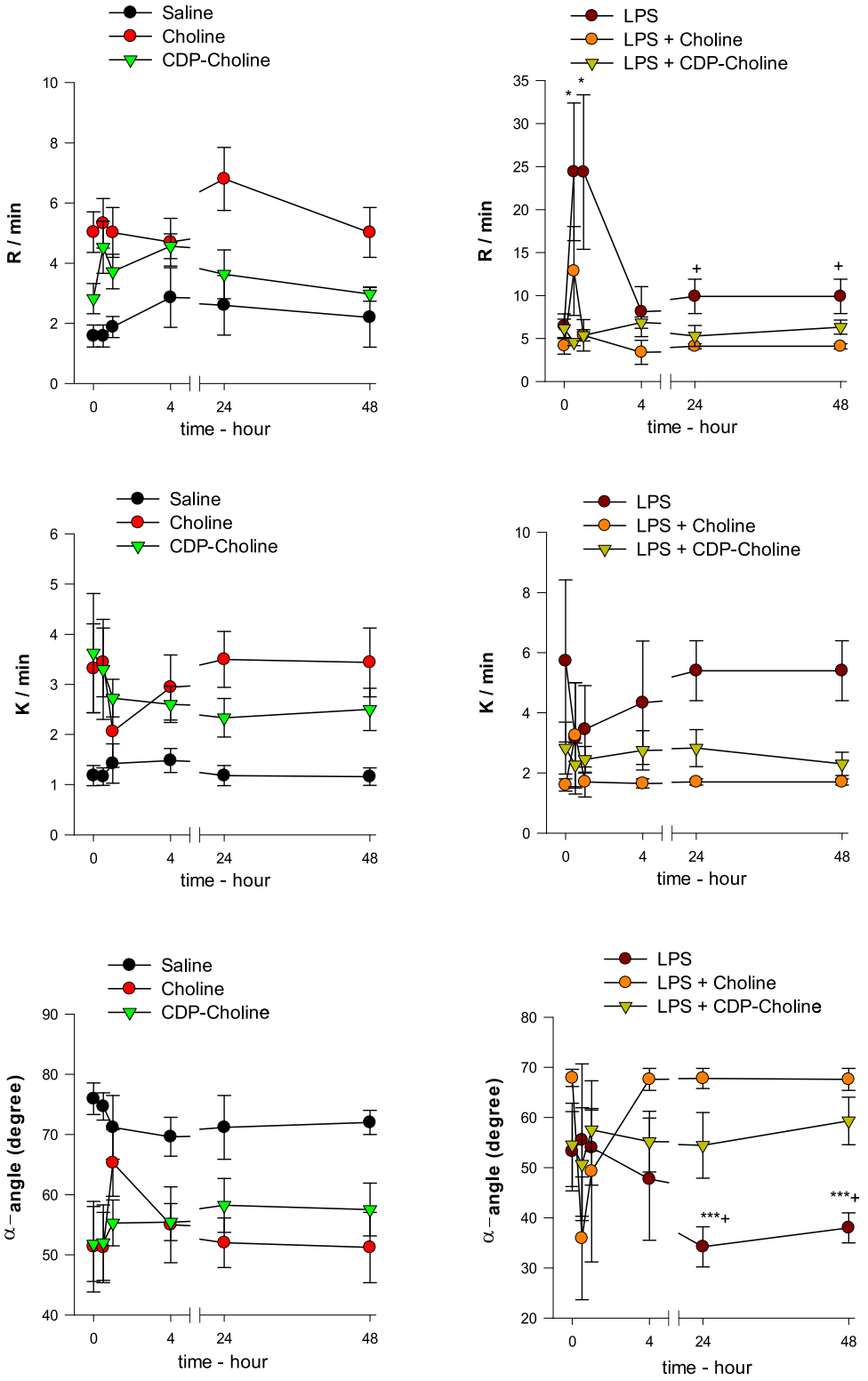

Fig. 1. Effect of choline or CDP-choline treatment on kaolin-activated thromboelastography (TEG) parameters (R: reaction time; K: kinetic time; and alpha angle) in dogs receiving normal saline, choline, or CDP-choline with or without endotoxin (LPS) administration. *$P < 0.05$, ***$P < 0.001$ compared to baseline (0 h); + P < 0.05 between groups.

TEG parameters used for the overall assessment of coagulation process included reaction/clotting time (R-time), kinetic time or clot formation time (K-time), rate of clot formation and streghtining (α-angle), clot strength (maximum amplitude – MA, and G value), as well as fibrinolytic parameters; estimated percentage of lysis (EPL), amplitude (A), and clot lysis at 30 min after maximum clot strenght (LY30). Hypercoagulable state was defined by shortened reaction time (R- and/or K-time), increased α-angle, and/or MA values; and hypo-coagulable state was defined by prolonged R- and K- time, and/or decreased α-angle and MA values (Müller et al., 2014). Hypofibrinolysis was defined by increasing LY30 value which was higher than that of the reference for healthy human (>7.5%) (Chapman et al., 2013), and dogs (>6.4%) (Langhorn et al., 2021).

2.5. Statistical analysis

Statistical analysis was performed using SigmaStat 3.5 software. Data were expressed as mean ± standard deviation. Holm-Sidak test was used for pairwise and control group comparisons, along with analysis of variance (ANOVA) for repeated measurements. Charts were created with SigmaPlot program (2000–6.1, SPSS Inc.).

3. Results

The results of the present study provide a well-characterized endotoxemia within half an hour just after intravenous endotoxin administration considering clinical and hemato-biochemical alterations such as

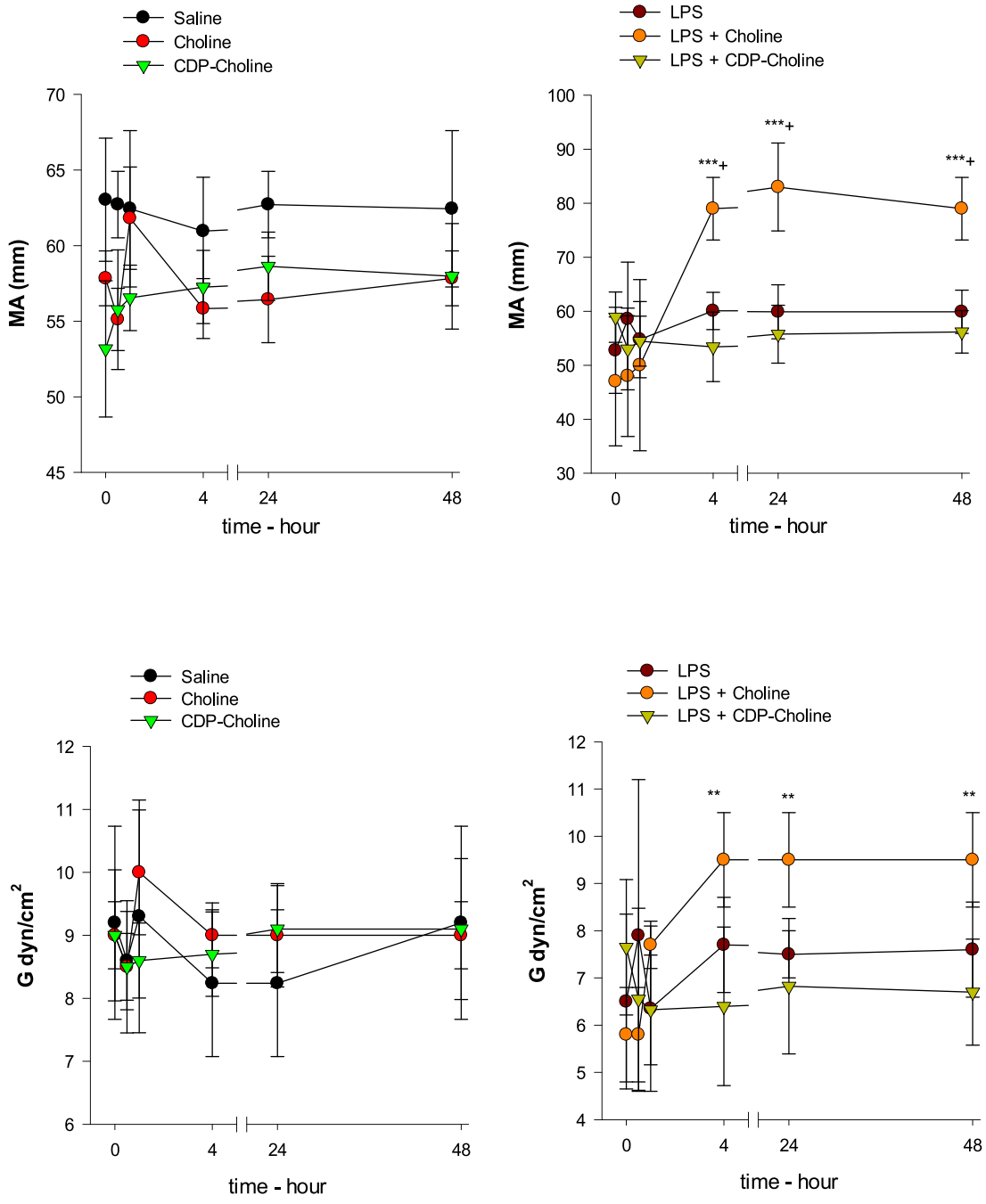

Fig. 2. Effect of choline or CDP-choline treatment on kaolin-activated thromboelastography (TEG) parameters (MA: maximum amplitude; and G value calculated by MA and R time) in dogs receiving normal saline, choline, or CDP-choline with or without endotoxin (LPS) administration. $P < 0.05$, ***$P < 0.001$ compared to baseline (0 h); + $P < 0.05$ between groups.

fever, increased heart and respiratory rates, leukopenia, thrombocytopenia, and increased serum levels of hepato-renal injury markers and an inflammatory marker CRP in dogs, which was reported in detail in our previous studies (Ilcol et al., 2005; Kocaturk et al., 2016; Kocaturk et al., 2021; Yilmaz et al., 2006). Treatment with choline or CDP-choline prevented or attenuated endotoxin-induced cardiovascular and respiratory alterations (Kocaturk et al., 2021), leukopenia and thrombocytopenia (Yilmaz et al., 2006) and the increase of hepato-renal injury markers (Ilcol et al., 2005). In our previous studies, we elaborately reported the effects of intravenous choline and cdp-choline treatments on clinical and hemato-biochemical parameters in dogs with endotoxemia (Yilmaz et al., 2005, 2006, 2008, and Yilmaz et al., 2010; Ilcol et al.,

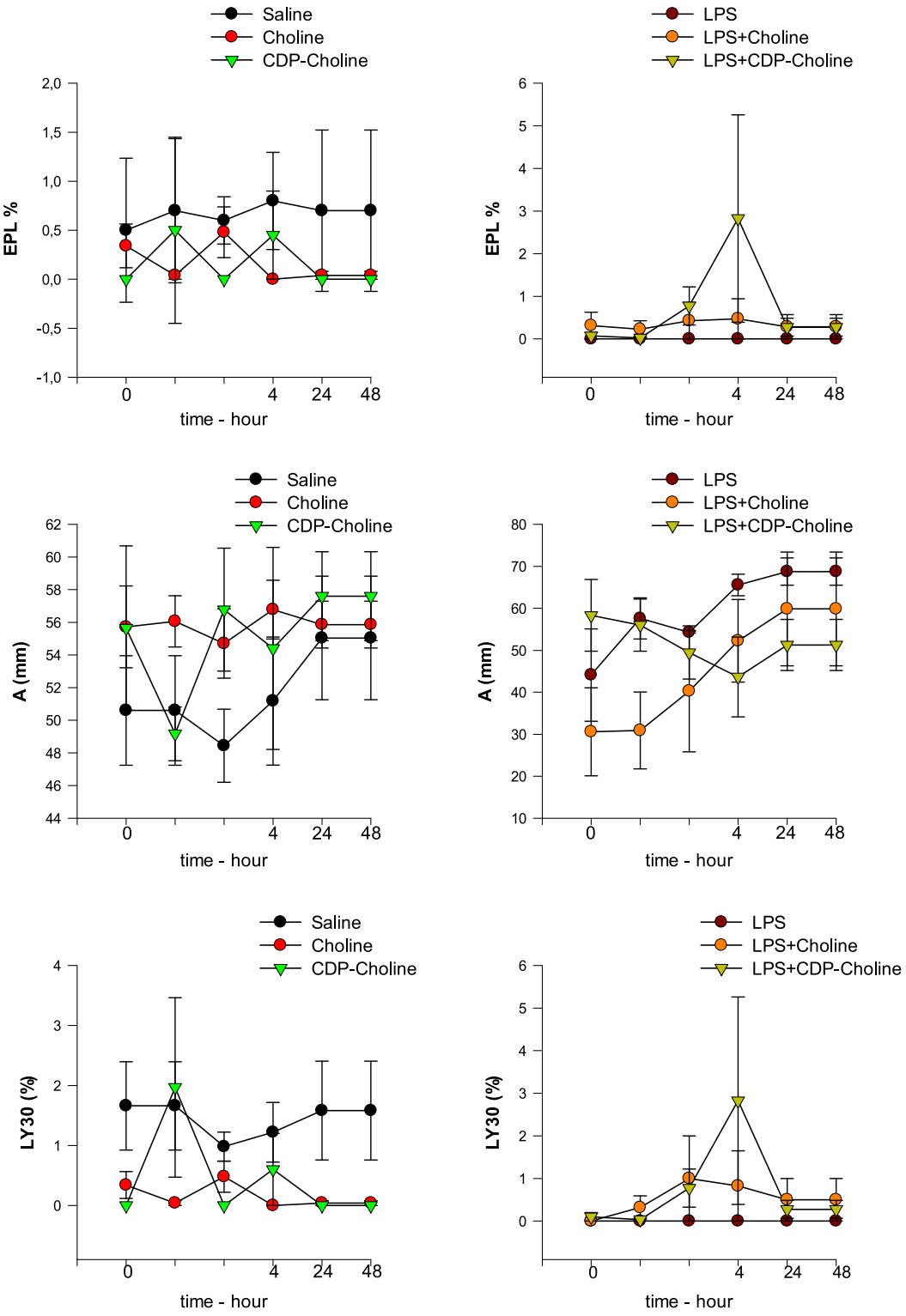

Fig. 3. Effect of choline or CDP-choline treatment on the fibrinolytic parameters of kaolin-activated thromboelastography (TEG) (EPL: estimated percentage of lysis; A value: amplitude, and LY30: Lysis time at 30 min after initial clot formation) in dogs receiving normal saline, choline, or CDP-choline with or without endotoxin (LPS) administration. These fibrinolytic parameters did not show statistically significant changes within and between groups.

2005; Ilcol et al., 2009; Eralp et al., 2011; Kocaturk et al., 2021). Thus, only the results of TEG measurements before and after choline or CDP-choline with or without LPS administarations were presented detailed in this study.

The changes in TEG parameters between and within groups were given in Fig. 1 (R-time, K- time, and α-alpha angle), Fig. 2 (MA and G values), Fig. 3 (EPL, A, and LY30 values), and Fig. 4 (CI value). Also, we provided numeric data of some TEG measurements in all groups (R time, K time, alpha angle, MA, and G values in Suplementry file 1–5, respectively).

When control groups were evaluated, among TEG parameters included in the study, eight TEG measurements didn't show any statistically significant changes in the saline, choline and CDP-choline groups, but CI values were lower ($P < 0.01$) at 24–48 h after choline treatment, compared to those of other groups.

LPS administration led to increases in R-time (at 0.5–1 h; $P < 0.05$), and A value (0.5–48 h; $P < 0.05$), and decreases in K-time (at 0.5 h; $P > 0.05$), alpha angle (at 24–48 h; $P < 0.001$) and CI values (at 24–48 h; $P < 0.01$), compared to their baselines. Following the LPS administration, initial increases in R time (24 ± 8.0 min) started to decrease from at 4th hr (8.1 ± 2.9 min) but thereafter was still higher (9.9 ± 2.0 min; at 24–48 h) than those of other treatment groups; 4.1 ± 0.3 min in LPS + Choline and 6.3 ± 0.8 min in LPS + CDP-Choline. Decreased K time returned to baseline value at 24th hr in LPS-treated dogs, whereas decreased alpha angle could not return the baseline and remained lower ($P < 0.001$) than those of choline groups. There were no statistically significant changes in some TEG parameters (MA, G, EPL, and LY30) at all sampling times compared to their baseline in the LPS group. In addition, following the LPS administration, TEG tracing changed from normocoagulable state to hypercoagulable state (Fig. 5A and B). Two dogs suffered from altered coagulation states, one dog from primary fibrinolysis, and one dog from hypocoagulable state, within 4 h after LPS administration (Fig. 5C and D).

In the groups of choline and CDP-choline treatments, the R value elevation in the first hour after the LPS administration was successfully prevented similar to each other by both treatments ($P < 0.05$). At 24–48 h sampling times, R value decreased but remained significantly higher ($p < 0.05$) compared to both choline groups. However, despite no significance between the groups at these times, treatments with either choline or CDP-choline kept the R value almost at the baseline level. Any significant changes between pre- and post-treatment values were seen in the measurements of K value in the treatment groups of choline and CDP-choline. Observed decreases in the α-angle at 24–48 h after LPS administration was not seen in the groups of choline treatments ($P < 0.001$). Changes of MA and G values were in a same tendency. All the G value is an exponential reflection of the MA measurement. MA and G values in LPS and LPS + CDP-choline groups did not show any statistically significant change during the study, whereas they showed an increase at 4–48 h in the LPS + Choline group compared to their baselines within the groups (at least, $P < 0.05$) and compared to those of the LPS, and LPS + CDP-choline groups ($P < 0.001$).

When the TEG parameters of clot stability and fibrinolysis were evaluated between the test groups, there was the same trend of increasing TEG A value after the LPS and LPS + Choline treatments, but this trend declined (not statistically significant level) in the LPS + CDP-Choline group. Choline treatment increased the CI value at 1–48 h compared to its baseline ($P < 0.01$) and to those of other treatment groups ($P < 0.01$). The control group mean CI results remained between 0 and + 1, whereas the endotoxemia group results dropped after the 0.5 h under 0 till −5 at 24 h and 48 h time points. Choline treatment compared to CDP-choline raised CI till +2, whereas CDP-choline only had an mild upregulating effect as seen in Fig. 4. Choline treatments protected the dogs from coagulation abnormalities detected by TEG, and normocoagulable state observed at baseline did not change during the study's period after choline administrations in the dogs receiving LPS (Fig. 5E and F).

4. Discussion

The present study shows that intravenous endotoxin (LPS) administration can cause alterations in the viscoelastic prosperities of the whole blood, including prolonged clotting time and diminished clot kinetic, clot strength, clot elasticity, and clot (fibrino)lysis. Our results provide novel information on the utility of intravenous choline or CDP-choline administration with regard to the inhibition of LPS-induced alterations in these viscoelastic properties of the blood clot measured by TEG in dogs. Such inhibition includes increase in R and K time, and decrease in alpha angle in TEG parameters by choline and CDP-choline. Compared to choline, CDP-choline exhibited a more pronounced effect in the protection of coagulation abnormalities including hyper/hypocoagulable state and hyperfibrinolysis in dogs with endotoxemia.

TEG provides overall assessment of coagulation process by measuring R-time (clotting time), K-time (clot formation time), α-angle (rate of clot formation and streghtining), MA and G (clot strength), as

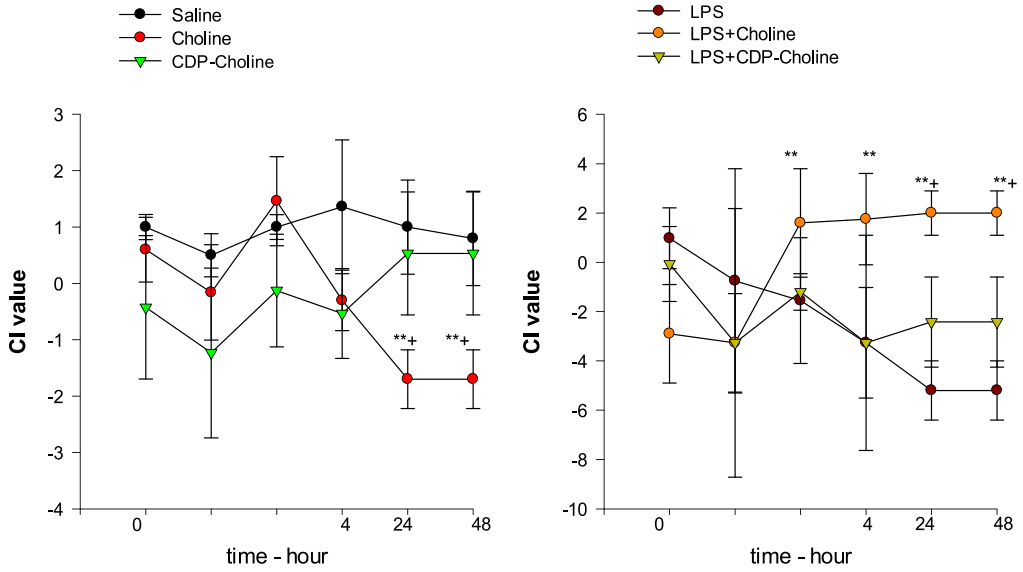

Fig. 4. Overall assessment of coagulation based on the coagulation index (CI), a calculated parameter from R, K, and MA values of TEG in dogs receiving saline, choline, and CDP-choline with or without LPS administration. *P < 0.05, ***P < 0.001 compared to baseline (0 h); + P < 0.05 between groups.

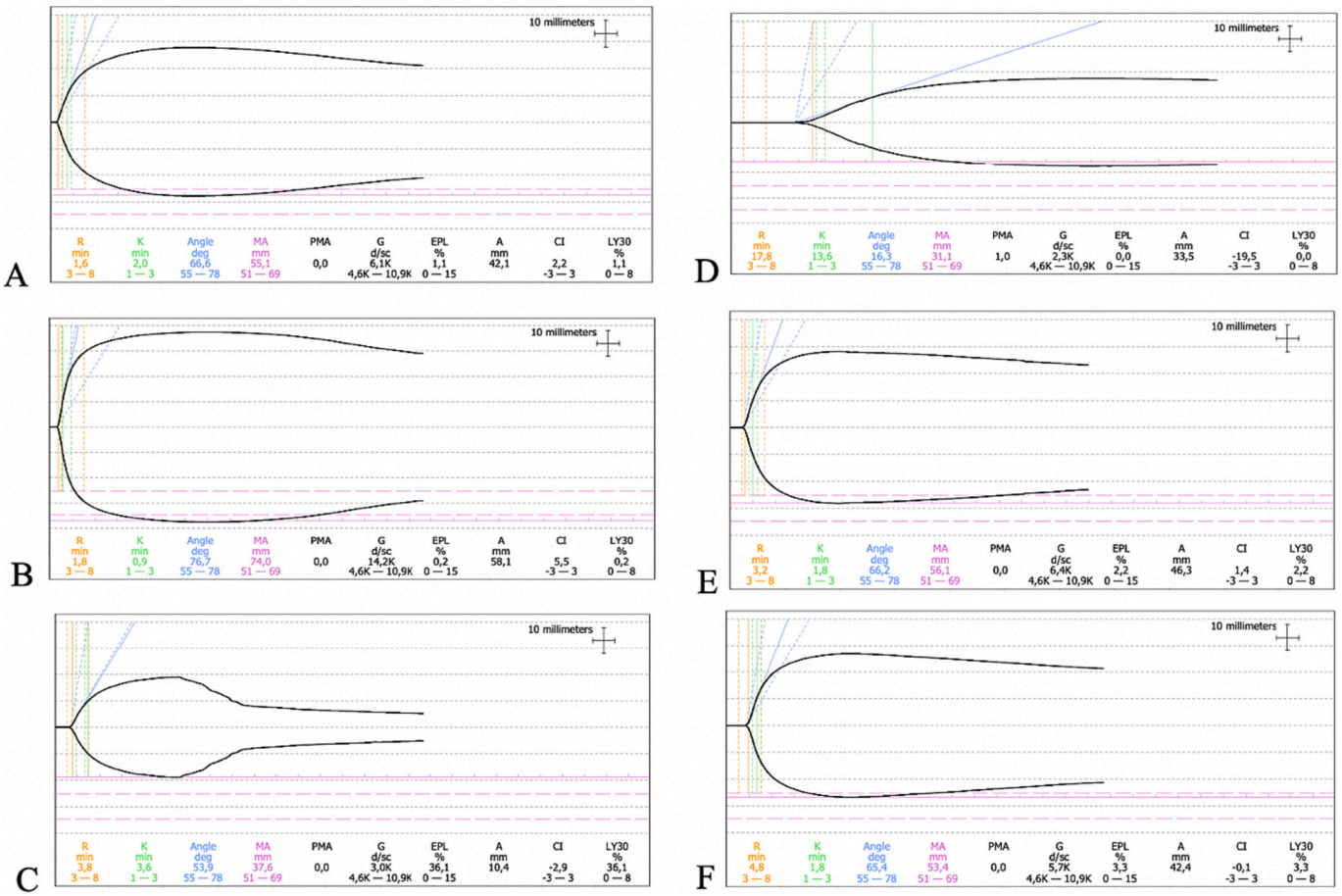

Fig. 5. Thromboelastogarphy (TEG) profiles from dogs receiving LPS; TEG tracing reflecting a normocoagulable state before treatment (A), TEG tracing reflecting a hypercoagulable state at 1 h after LPS (B), TEG tracing reflecting a primary fibrinolysis state at 4 h after LPS (C), and TEG tracing reflecting a hypocoagulable state at 4 h after LPS (D). E and F represent normocoagulable states before and 4 h after LPS + CDP-choline treatment.

well as fibrinolytic parameters (EPL, A, and LY30). In the present study, TEG measurements didn't show any statistically significant change in the control (saline, choline and CDP-choline) groups, but LPS led to alterations in some TEG parameters. The R value, reflects the time of the beginning the test till a preset (2 mm wide trace) fibrin formation, which is primarily affected by anticoagulants, inhibitors, and coagulation factors (Bauer et al., 2009). In the present study, following the LPS administration, initial increases in R time started to decrease from 4 h but thereafter was still higher at 24–48 h than its baseline, and the reference values published for dogs (Bauer et al., 2009). In contrast, in the previous study (Eralp et al., 2011), R-time did not show statistically significant changes throught 48 h after LPS treatments in dogs. This discrepancy could be explained by using the different dosages of LPS (0.1 mg/kg *vs* 0.02 mg/kg) in those studies. According to a ROTEM study, an influence of PLT count on clotting time, equivalent to R time, was detected (Bolliger et al., 2012). Thus, prolonged R time (in other means prolonged clotting time) may be related with low PLT count and loss of coagulation factors, both representing the presence of consumptive coagulopathy (DIC), in dogs with endotoxemia. This explanation may be supported by the findings in which following the LPS administration within 0.5 h, a fast coagulation was triggered, and consumptive coagulopathy was detected based on the thrombocytopenia and loss of coagulation factors (II, VII, VIII, IX, XI, XII, and XIII), as reported in our previous studies (Yilmaz et al., 2010; Eralp et al., 2011). Therefore, observed prolongation of R time may be used as one of the diagnostic indicators for the consumptive coagulopathy during endotoxemia.

TEG K or kinetic value did not show statistical changes during the study in accordance with the results of our previous study (Eralp et al., 2011), but herein there was a decreasing trend of the K value within the first hour after LPS administration. In human septic patients with hypo- or hyper-coagulative profiles, generally at least 3 TEG parameters were considered and K-value was always one of them (Ninan et al., 2021; Kim et al., 2021). Whereat naturally occurring sepsis and disease's pathologies can vary in contrast to experimental investigations in human and most likely in animals (van Lier et al., 2019). Despite the close relation between K value and α-angle measurements, there was a significant change in only α-angle results; lower at 24–48 h ($P < 0.001$) after LPS compared to its baseline. Since the α-angle is a measure of the speed at which fibrin builds up and cross-linking occurs, observed decreases in α-angle in this study may be associated with the impaired fibrin-PLT interaction and cross-linking, as well as decreasing fibrinogen level (Bauer et al., 2009; Bose and Hravnak, 2015) and low PLT counts to a lesser extent (Bauer et al., 2009). This explanation may be supported by the presence of hypofibrinogemia and/or thrombocytopenia occurred at 1–12 h after LPS administration in dogs (Eralp et al., 2011; Yilmaz et al., 2010).

There were no statistically significant changes in some TEG parameters relating with the clot strength (MA and G value) and clot lysis (EPL, A, and LY30) between pre- and post-treament time points in the LPS group, in agreement with the results of our previous study (Eralp et al., 2011). Changes in TEG MA and G values after LPS were not as expected in this study. Since MA value is influenced by PLT count and function about 80% (Wiinberg et al., 2005), it could have been decreased in dogs with thrombocytopenia in this study. This unexpected result may be associated with increasing PLT function in response to low PLT count in

dogs with endotoxemia (Yilmaz et al., 2008). There was an increasing or decreasing trend in TEG A or CI values after LPS administration, respectively, which may be associated with a coagulapthy and low fibrinolysis in dogs receiving LPS alone. In parallel to this observation, TEG software tracings indicated a hypocoagulable state within 4 h after LPS administration in one dog.

In the present study, alterations observed in the TEG parameters were inhibited or attenuated by choline or CDP-choline treatment in dogs with endotoxemia. In addition, CDP-Choline had a more pronounced effect compared to choline with regard to protection from prolonged R time, shortened K time, and increased α-angle which were observed at different time points after the LPS administrations. Hypercoagulable state in TEG is defined by shortened reaction time (R- and/or K-time), increased α-angle, and/or MA values; whereas hypocoagulable state is defined by prolonged R- and K- time, and/or decreased α-angle and MA values (Ninan et al., 2021; Kim et al., 2021; Müller et al., 2014). Our results showed that the beneficial effects of choline treatments might have also resulted from the protection of hyper- or hypocoagulable states in the case of endotoxemia in dogs.

Several mechanisms may underlie beneficial effects of choline treatment in terms of LPS-induced coagulation abnormalities in dogs with endotoxemia. We previously showed a variety of positive effects of choline or CDP-choline treatment on endotoxemia basically depending on prevention of thrombocytopenia and suppression of cytokines (Yilmaz et al., 2006; Yilmaz et al., 2010), which would explain the normal R times in the CDP- choline and choline treatment groups in contrast to prolonged R times in endotoxemia. Endogenous and exogenous choline can suppress the cytokine expressions by activating the cholinergic anti-inflammatory pathway (Tracey, 2007; Yilmaz et al., 2010), resulting in the inhibition of Tissue Factor (TF) expression which is a key factor for excessive triggering of coagulation. The TF expression on monocytes and endothelial cells by proinflammatory cytokines in septic disorders may lead to consumption of coagulation factors (Tracey, 2007; Fay, 2010), which was the suggested as the main reason for prolonged R times in the endotoxemia group. Another possible reason for the beneficial effects of choline treatment on LPS-induced coagulation abnormalities may include the inhibition by choline of CRP, HGMB-1 and TNF-α in dogs (Yilmaz et al., 2008; Kocaturk et al., 2021; Xu et al., 2015; Liu et al., 2015). The effect of treatment on α-angle could be attributed to the inhibition of thrombocytopenia, as mentioned above and hypofibrinogenemia. Reported protection of the liver by choline in endotoxemia in dogs suggested the inhibition of fibrinogen depletion resulting in normal α-angle values (Ilcol et al., 2005; Kocaturk et al., 2021; Yilmaz et al., 2005). The liver has a pivotal role in coagulation, due to production of Vitamin K-dependent coagulation proteins including PC, PS, FVII, FIX and FX (Tracey, 2007). The primary fibrinolysis observed in a dog (Fig. 5C), with elevated LY30 may have resulted from a decreased hepatic clearance of tissue plasminogen activator (tPA), one of primary fibrinolysis reasons (Franchini et al., 2021). We previously showed that choline treatment prevented endotoxin-induced changes in serum biochemical markers associated with liver functions by improving tissue perfusion and restoring hypotension (Kocaturk et al., 2021; Yilmaz et al., 2005), thus, our case(s) may be prevented from the decrease of functioning coagulation proteins and excess tPA in circulation in choline-treated dogs with endotoxemia.

In the present study, among TEG parameters measured, following the choline treatment, only TEG MA variable reflecting peak clot rigidity was found to be altered in dogs with endotoxemia (LPS + Choline group). Although choline or CDP-choline cannot change the MA value at statistically significant levels during the study in healthy dogs, choline but not CDP-choline increased the MA value in dogs with endotoxemia. Observed increases in MA value may be associated with the increased clot rigidity or clot strength as a risk for thromboembolism during endotoxemia. This finding seems likely to raise concerns if and whether cardiovascular risks such as thrombus, stroke, and atherosclerotic plaques occur by excessive consumption of choline containing food or supplements. This discussion is mostly about the metabolites such as trimethylamine-N-oxide (TMAO) produced by intestinal microbiota after oral intake of choline products (Papandreou et al., 2020). It is unclear whether these side effects would be observed after intravenous choline administration and if it's the same for dogs at all. Whether the side effects mentioned for the clinical use of choline in dogs are valid for this species should be supported by future studies. However, it has been shown that the side effects that may occur during the use of choline can be quickly detected by treatment monitoring with TEG and can provide the opportunity to change treatment.

In fact, it can be monitored as a summary in the CI results that the effects of choline on MA are not observed in CDP-choline treatment. CI is a measurement to assess the overall coagulation status, calculated by a formulation based on R, K, MA and alpha results of the performed test. The human reference range lies between +3 and − 3. Results above +3 indicate hypercoagulability, while values below −3 indicate coagulopathy (Shaydakov et al., 2023). The standard deviations from the mean of zero in dogs is unknown. The clinical usefulness of CI variable has not been validated yet and is not included in routine evaluations in human and veterinary medicine (Shaydakov et al., 2023). The trend to a coagulopathy in endotoxemia was inhibited by choline, but the rise of CI by choline which may well reflect a safe positive effect, should be viewed with suspicion due to the lack of reference range. Furthermore, a tendency to hypercoagulation could be the case while interpreting the data with MA parameter and in comparison with the saline control group. Here, the importance of TEG's ability to evaluate coagulation as a whole, its ability to provide rapid results for monitoring inconsistent conditions, and its use in complex coagulation mechanisms becomes evident.

In conclusion, our results showed that 1) TEG may be used to describe the changes in viscoelastic properties of blood clot in response to endotoxemia, 2) intravenous choline or CDP-choline treatment seemed to be safe based on the TEG parameters in healthy dogs, 3) CDP-choline may be superior over choline in preventing dogs from LPS-induced coagulation abnormalities, and 4) intravenous choline treatment might be associated with a side effect as increased TEG MA value (increases in clot strenght) in dogs with endotoxemia. Practitioners should also be very careful if they decide to administer choline through intravenous route because of its potential side effects related with muscarinic cholinergic activation such as diarrhea, vomiting and salivation.

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CRediT authorship contribution statement

Oya Eralp Inan: Data curation, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Meric Kocaturk:** Data curation, Investigation, Methodology, Project administration, Writing – original draft. **Mehmet Cansev:** Conceptualization, Methodology, Writing – original draft. **Yesim Ozarda:** Formal analysis, Methodology, Writing – original draft. **Zeki Yilmaz:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Ismail Hakki Ulus:** Conceptualization, Project administration, Resources, Writing – original draft, Writing – review & editing.

Declaration of competing interest

There are no conflicts of interest.

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