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Evaluation of differential effects of CDP-choline and choline on parasympathetic activity and changes in choline levels with heart rate variability

Hasan KAZDAGLI[1], Suheda ALPAY[2], Hasan Fehmi OZEL[3], Elif BARIS[4]

[1] Department of Medical Services and Techniques, Vocational School of Health Services, Izmir University of Economics, Izmir, Turkey
[2] Department of Physiology, Faculty of Medicine, Manisa Celal Bayar University, Manisa, Turkey
[3] Department of Medical Services and Techniques, Vocational School of Health Services, Manisa Celal Bayar University, Manisa, Turkey
[4] Department of Medical Pharmacology, Faculty of Medicine, Izmir University of Economics, Izmir, Turkey

Corresponding Author: Elif BARIS
E-mail: elif.baris@ieu.edu.tr

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ABSTRACT

Objective: Heart rate variability (HRV) is used to evaluate the autonomic activity of heartbeat. This study aimed to investigate the effects of cholinomimetic drugs cytidine diphosphate-choline (CDP-choline) and choline, on short-term HRV parameters.

Materials and Methods: Animals were randomized into three groups; control (0.9% NaCl), choline (100 mg/kg), CDP-choline (400 mg/kg). Electrocardiography recordings were obtained for 45-minutes after treatments with 15-minutes intervals. HRV analyses and total choline level measurements in serum and heart tissues were performed.

Results: High frequency power and total power increased in treatment groups, while heart rates were decreased. Low frequency was decreased with choline while very low frequency power decreased with CDP-choline. Choline affected most of the HRV parameters in the first 15 minutes, while the effect of CDP-choline started within 30 minutes. Total choline levels were higher in both treatment groups than in the control while the levels were also higher in the choline group compared to CDP-choline group.

Conclusion: This study showed that CDP-choline and choline treatments produced a rapid response to short-term HRV parameters, while increasing tissue choline levels. Moreover, the differences in effects and onset time between the drugs on HRV might be related to tissue choline concentration.

Keywords: Parasympathomimetics, Autonomic nervous system, Cytidine diphosphate choline, Choline

1. INTRODUCTION

Complicated patterns of variability in biological systems have been widely investigated in various areas of medical research. Multifarious oscillations in time differences between successive beats and heart rhythm cause rapid reactions in the cardiovascular system resulting from different stressors that affect homeostasis [1]. Heart rate variability (HRV) is defined as the differences in duration between successive heartbeats in electrocardiographic (ECG) recording [2]. HRV measures the function of the autonomic nervous system (ANS) as well as the individual contributions of the sympathetic (SNS) and parasympathetic nervous systems (PNS). Since, the nervous vagus is the key contributor of the PNS, evaluating vagal activity with HRV analyses is used for assessment of changes in parasympathetic activity [3].

Apart from the normal function, pharmacological interventions affecting the autonomic nervous system and their effect on cardiac dynamics can also be analyzed by using HRV analysis. Choline, a precursor of the main neurotransmitter in parasympathetic nervous system acetylcholine (ACh) also directly interacts with cholinergic receptors to induce cholinergic neurotransmission [4]–[6]high mobility group box 1 (HMGB1. CDP-choline is an intermediary endogenous molecule that is produced during phosphatidylcholine (PC) synthesis via Kennedy pathway [7]. Phosphodiesterases (PDEs) in the cell membrane split CDP-Choline into Choline and cytidine, which raises the total choline levels in the brain and circulation [8], [9]. Choline rapidly diffuses into the blood circulation, cross the blood–brain barrier and plays an important part in acetylcholine synthesis [10].

Choline and CDP-choline have shown potential clinical use in various conditions. Abnormal choline metabolism has been observed in various types of cancer, and it has been detected using magnetic resonance spectroscopy (MRS) approaches [11]. CDP-choline has been studied in various neurological disorders, including traumatic brain injury, Alzheimer's

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disease, Parkinson's disease, learning and memory disorders, amblyopia, acute ischemic stroke and glaucoma [12-14]which is identical to the natural intracellular precursor of phospholipid phosphatidylcholine. Following injection or ingestion, citicoline is believed to undergo quick hydrolysis and dephosphorylation to yield cytidine and choline, which then enter the brain separately and are used to resynthesize CDP-choline inside brain cells. Neuroprotective activity of citicoline has been repeatedly shown in preclinical models of brain ischaemia and trauma, but two recent, large, pivotal clinical trials have revealed no benefits in ischaemic stroke and traumatic brain injury. However, the substance seems to be beneficial in some slowly advancing neurodegenerative disorders such as glaucoma and mild vascular cognitive impairment. This paper critically discusses issues related to the clinical pharmacology of citicoline, including its pharmacokinetics/biotransformation and pharmacodynamics/mode of action. It is concluded that at present, there is no adequate description of the mechanism(s. These studies have shown some beneficial effects of CDP-choline, with rare side effects such as stomach pain, diarrhea, and headaches [14].

Besides, its cholinergic interactions via Choline moiety, purinergic receptors has been shown to contribute some of the effects of cytidine moiety of CDP-Choline [15]1.0 and 2.0 µmol which might be responsible for its distinct effects from Cho. However, the differential effects of Choline and CDP-Choline on HRV has not been established yet. The present study investigated the differential effects of CDP-Choline and Choline on parasympathetic nervous system activity via evaluating short-term HRV parameters together with the total choline/ACh levels.

2. MATERIALS and METHODS

Experimental Groups and Heart Rate Variability Analyses

The regional Ethics Committee for Animal Experiments authorized the experimental study (No: 77.637.435/224). Adult male (12-16 weeks old) wistar rats (310 ± 22, 45 g, n=24) were used for the experiments [16]. The animals were kept in the animal care center under ad libitum conditions for at least five days prior to the start of the experiments in 12 hours dark/light cycle, at 20-22 °C. Ketamine/Xylazine (75/15 mg.kg-1, Sigma-Aldrich, PHA568487) was administered intraperitoneally (i.p.) for anesthesia before the operation [17]. Pedal pain reflex and respiration frequency were monitored to ensure the level of anesthesia after the injection.

To assess the impact of Choline and CDP-Choline on the HRV and heart rate complexity, 24 rats were randomized into the three groups (n=8): (i) Control (0.9% sodium chloride (NaCl)), (ii) choline (100 mg/kg) and (iii) CDP-Choline (400 mg/kg) [18], [19]. Choline chloride (C7017, Sigma Aldrich), CDP-choline sodium salt (C0256, Sigma Aldrich) or 0.9% NaCl injections were administered intraperitoneally (i.p.) after the baseline measurements. For ECG recordings, needle electrodes for Lead II were placed on right arm and left leg [20]. ECGs were recorded using Powerlab/SP8 (ADInstruments, Australia). LabChart 7 software (ADInstruments, Australia) was employed for R wave detection.

R waves were detected automatically via the Pan-Tompkins real-time QRS recognition software and a tachogram of RR intervals was created [21]. These RR tachograms were converted to time series using Berger interpolation. For all HRV analyses, Kubios HRV Software (University of Eastern Finland) was used. HRV analysis was conducted in three domains: (i) Time Domain Analyses; root mean square of successive deviations between regular heartbeats (RMSSD), Standard deviation of NN intervals (SDNN), Baseline width of the RR interval histogram (TINN), the stress index (SI), and mean heart rate (HR), (ii) Frequency Domain Analyses; High Frequency (HF), Low Frequency (LF), Very low frequency (VLF), and LF/HF ratio and (iii) Nonlinear Analyses; Detrended Fluctuation Analysis (DFA) [22], Poincaré plot analysis [23] and Entropy Analysis [24] as we described earlier [25].

Choline measurements

A commercially available kit was used to measure the total choline/ACh levels in the serum and cardiac tissues (Sigma-Aldrich, MAK056). Serum samples were obtained with serum-separator collection tubes and centrifuged immediately at the end of the protocol. Homogenates were prepared with the 10 mg of total heart tissues and lysed with choline assay buffer on ice. Total choline/ACh levels were determined by spectrophotometer according to the instructions [18].

Statistical Analysis

The Shapiro-Wilk test was employed to analyze normal data distribution. One-way ANOVA and post hoc Tukey–Kramer tests were used for comparisons between groups (GraphPad Prism 5, La Jolla, CA). Data were presented as mean ± standard error of the mean (S.E.M.) and $P<0.05$ was considered statistically significant.

3. RESULTS

Heart Rate Variability; Frequency Domain, Time Domain and Non-Linear Analyses

Time domain analysis showed mean HR, RMSDD and SI between the time periods (baseline,15th minute, 30th minute and 45th minute) after the drug injection did not significantly change in the control group ($P>0.05$). In choline-treated group mean HR and SI were significantly lower at 15th minute, 30th minute and 45th minute while SDNN, RMSDD, TINN were significantly higher after the injection compared to baseline measurements (*: $p<0.05$; **:$p<0.01$; ***: $p<0.001$). In CDP-choline-treated group mean HR were significantly decreased within 15th minutes while SDNN and RMSDD were significantly elevated at 30th minute after the injection compared to baseline measurements (†: $P<0.05$; ††:$P<0.01$) (Figure 1).

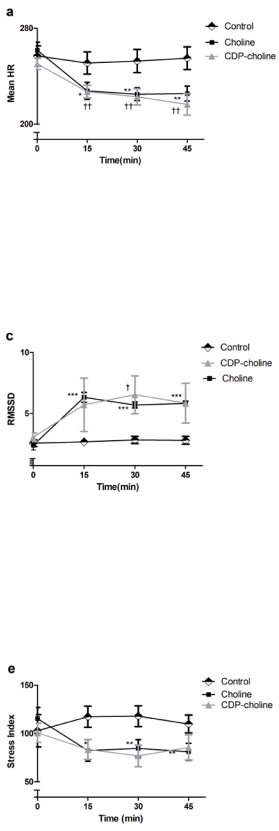

Figure 1. Time domain parameters of experimental groups. *Mean heart rate (HR) (a), Standard deviation of normal intervals (SDNN) (b), Root mean square of successive RR interval differences (RMSSD) (c), Baseline width of the RR interval histogram (TINN) (d) and Stress Index (e). One-way analysis of variance (ANOVA) with post hoc Turkey-Kramer multiple comparison tests were used for statistical analysis. Data were shown as mean and S.E.M. (n=8 per group). (*); P<0.05, (**); P<0.01, (***); P<0.001 vs. baseline measurements of choline group, (†); P<0.05, (††); P<0.01 vs. baseline measurements of CDP-choline group.*

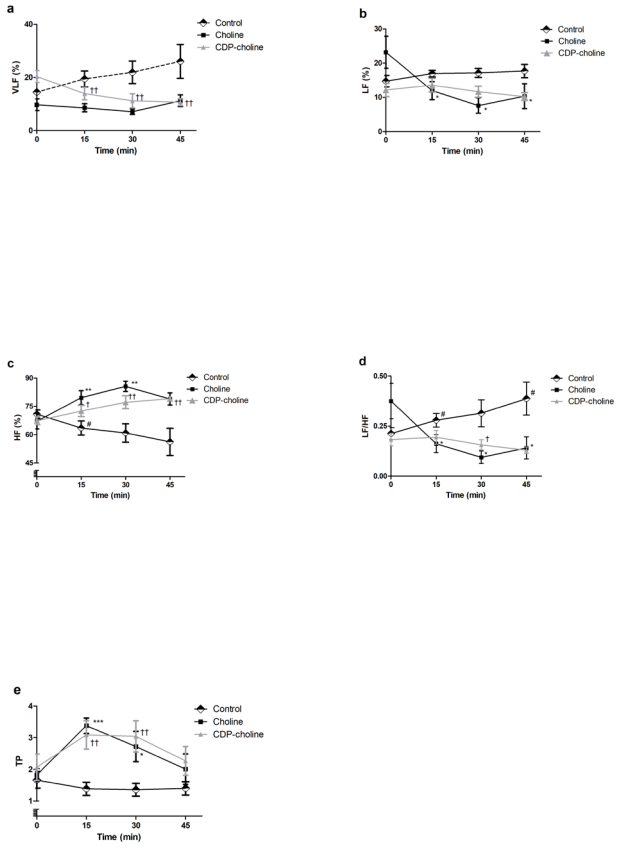

Figure 2. Frequency domain parameters of experimental groups. *Very low frequency (VLF) (a), low frequency (LF) (b), high frequency (HF) (c), LF/HF ratio (d) and total power (TP) (e). One-way analysis of variance (ANOVA) with post hoc Turkey-Kramer multiple comparison tests were used for statistical analysis. Data were shown as mean and S.E.M (n=8 per group). (*); P<0.05, (**); P<0.01, (***); P<0.001 vs. baseline measurements of choline group, (†); P<0.05, (††); P<0.01 vs. baseline measurements of CDP-choline group.*

Regarding the frequency domain analysis; changes in mean relative powers of LF and HF, and TP between the time periods (15th minute, 30th minute and 45th minute) did not significantly change compared to baseline measurements in control group (P>0.05). In Choline-treated group mean LF and LF/HF ratio were significantly lower in 15th minute, 30th minute and 45th minute while HF and TP were significantly higher after the injection compared to baseline measurements (*: P<0.05; **: P<0.01; ***: P<0.001). In CDP-choline-treated group LF and LF/HF ratio were significantly lower in 15th minute, 30th minute and 45th minute while HF and TP were higher after the injection compared to baseline measurements (†: P<0.05; ††: P<0.01) (Figure 2).

Regarding the frequency of nonlinear analysis; changes in mean Sample Entropy (SampEn) between the time periods of baseline measurements, 15th minute, 30th minute and 45th minutes after the operation did not significantly change in control and choline groups. In CDP-choline group mean SampEn were significantly higher in 45th minute compared to baseline measurements (P<0.05; Figure 3e). The changes in mean differences of $DFA\alpha_1$ significantly increased in control group compared to baseline measurements at 30th and 45th minutes in CDP-choline group (P<0.05). In Choline group mean $DFA\alpha_1$ were significantly decreased in 30th minute (P<0.05). $DFA\alpha_2$ did not significantly change in the groups ($DFA\alpha_1$: P<0.05, $DFA\alpha_2$ P<0.01; Figure 3f-g). SD1 and SD2 parameters did not significantly change in the control group compared to baseline measurements. In Choline and CDP-choline-treated groups mean SD1 significantly increased while SD2 and SD2/SD1 ratio significantly decreased

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in 15, 30 and 45 minutes after the injection, compared to baseline measurements (P<0.001). (Figure 3a-c).

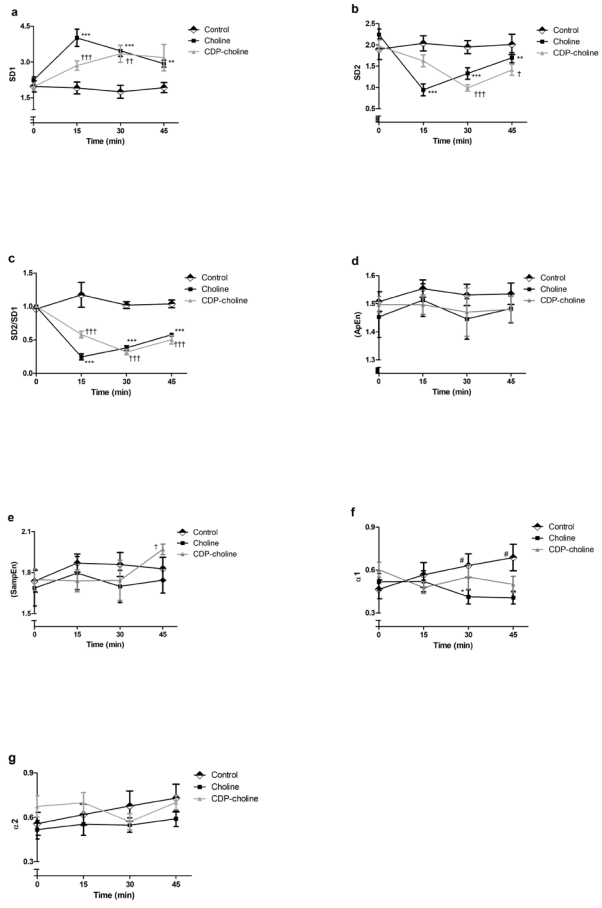

Figure 3. Non-linear parameters of experimental groups. *Standard derivation 1 (SD1) (a), standard derivation 2 (SD2) (b), SD2/ SD1 ratio (c), Approximate Entropy (ApEn) (d), Sample Entropy (SampEn) (e), short-term Detrended Fluctuation Analysis (DFAα1) (f) and long-term Detrended Fluctuation Analysis (DFAα2) (g). One-way analysis of variance (ANOVA) with post hoc Turkey-Kramer multiple comparison tests were used for statistical analysis. Data were shown as mean and S.E.M (n=8 per group). (*); P<0.05, (**); P<0.01, (***); P<0.001 vs. baseline measurements of choline group, (†); P<0.05, (††); P<0.01 vs. baseline measurements of CDP-choline group.*

Total Choline Measurements

Total choline/ACh levels in serum samples increased in Choline (2.5 ± 0.03 nM) and CDP-choline(2.3 ± 0.02 nM) treated groups compared to control group (0.5 ± 0.0005 nM; P<0.001; Figure 4a). The levels in heart homogenates increased in Choline(3.7 ± 0.08 nM) and CDP-choline(3.3 ± 0.04 nM) treated groups compared to control group (1.2 ± 0.01 nM; P<0.001; Figure 4b). Total choline/ ACh was significantly higher in serum and heart tissues of Choline-treated group compared to CDP-Choline groups (P<0.001 and P<0.01 respectively).

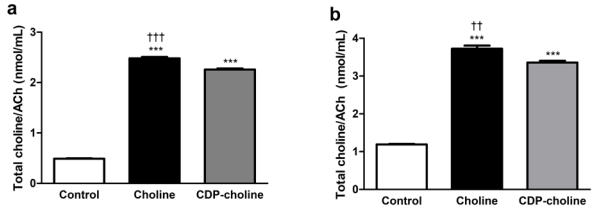

Fig 4. Total choline/acetylcholine levels of experimental groups. *Total choline/ACh levels of serum (A) and heart (B) tissues of experimental groups. One-way analysis of variance (ANOVA) with post hoc Turkey-Kramer multiple comparison tests were used for statistical analysis. Data were shown as mean and S.E.M (n=8 per group). (***); P<0.001 vs. control group, P<0.01, (†††); P<0.001 vs. CDP-choline group.*

4. DISCUSSION

Heart rate variability is a valuable tool to evaluate the ANS activity, however, effects of parasympathomimetic drugs on HRV analysis have not well established. In present study, our aim was to evaluate effects of parasympathomimetic drugs CDP-choline and choline on HRV parameters of time domain, frequency domain and nonlinear analyses. This study's primary findings can be summarized as follows: (i) Choline and CDP-choline treatment significantly changed HRV parameters that indicated parasympathetic system activation (ii) Effects of choline treatment affected most of the HRV parameters in first 15 minutes, whereas the effects of CDP-choline started within 30 minutes after the injection (iii) total choline/ACh levels in heart and serum tissues were markedly raised by choline and CDP-choline injection, and they were also significantly higher in choline group compared to the CDP-choline group.

Choline treatment has been shown to produce a significant reduction in mean heart rate without any significant change after the CDP-choline treatment in dogs monitored with 2 hours periods. The differences were considered as possibly related with choline's direct agonistic effect on muscarinic receptors along with elevation of the vagal tone by stimulating acetylcholine synthesis within the heart although not directly evaluated in scope of the study [26]. Accordingly, our data showed that choline and CDP-choline significantly changed mean heart rate starting from 15th minutes after the injection compared to baseline values while increasing HF and decreasing LF/HF ratio reflecting elevation in parasympathetic activity. During the monitoring period, we observed that choline and CDP-choline did not exhibit significant differences within the first 45 minutes after the injections. However, it is worth noting that CDP-choline treatment did not lead to any changes in the LF parameters, which reflected sympathetic activation. On the other hand, choline treatment resulted in a significant decrease in LF. This variance in LF levels might account for the slightly different effects we observed in our current findings. We attribute this outcome to the extended monitoring periods and the evident inhibitory influence of choline on sympathetic activity.

Furthermore, we noticed a positive chronotropic effect, which is typically induced by a sudden disruption in the balance between sympathetic and vagal nerves. This results in the predominance of the parasympathetic nervous system in controlling heart functions. As seen in the changes in mean heart rate following vagal activation, our HRV analysis also suggested shifts in the sympathovagal balance towards the parasympathetic nervous system.

CDP-choline is an endogenous intermediate [7] molecule that can be metabolized to choline and cytidine [10]. As a result, choline levels in blood circulation increase and cross the blood brain barrier to contribute acetylcholine synthesis which produce its therapeutic effects in many diseases affecting vascular systems within the body including such as stroke and brain injury [27-30]. Exogenously delivered CDP-choline and choline increase total choline levels in the brain and blood circulation [8, 9, 31]. An earlier study found that intraperitoneal injection of CDP-choline or phosphocholine at equal doses results in significant increases in serum free choline concentrations, which have been shown to cause significant hyperglycemia due to increase in cholinergic neurotransmission [32]. Increase in circulating choline concentrations (ie > 500 μM) has been shown to induce bronchoconstriction by activating PNS. The differences between choline and CDP-choline were possibly related with choline's direct agonistic effect on muscarinic receptors at higher concentrations [26]. Our data indicated that single dose of choline injection elevated plasma total choline/acetylcholine levels significantly compared to CDP-choline which might be responsible for the distinct onset times of effects on HRV parameters.

In present study, HRV parameters indicating parasympathetic activity, RMSSD, HF, and DFAα1, significantly changed after both choline and CDP-choline injection. These parameters, by their nature, reflect relatively fast changes in heart rate time series. Our data indicated that both choline and CDP-choline treatments affect short term HRV parameters and fast changes in heart rate time series within 45-minutes.

HRV parameters of sympathovagal balance including LF/HF ratio and SD2/SD1 ratio decreased after choline and CDP-choline injection indicating parasympathetic system activation and changes in relatively faster oscillations in heart rate time series. However, LF/HF ratio also increased in control group which may be due to the effects of Ketamine/Xlazyne anesthesia [25] although, significant changes between treatment groups and controls indicate prominent parasympathetic system activity with the cholinomimetic drug administrations.

Conclusion

Our results showed, choline and CDP-choline treatments produce a rapid response on short-term HRV parameters related with parasympathetic system activity via increasing total choline/acetylcholine levels in serum and heart tissue while the onset time of effect might differ between them.

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Compliance with the Ethical Standards

Ethics Committee approval: This study was approved by Manisa Celal Bayar University, Institutional Ethics Committee for the Care and Use of Experimental Animals (approval no:77.637.435/224).

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Conflicts of interest/Competing interests: Authors state no conflict of interest.

Authors contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission. HK, SA, HFO and EB: Designed and conducted the experiments, HK and EB: Wrote the draft manuscript. All authors reviewed the final manuscript.

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