Physiology and Pharmacology

Physiol Pharmacol 23 (2019) 59-69 **www.phypha.ir/ppj**

Original Article

Transient modulation of hydroxyzine, an antihistamine and anxiolytic agent, on the cardiac autonomic activity in healthy subjects

Fateme Rezaeyanzade[1], Nematollah Ahangar[2], Abbas Alipour[3], Ali Ghaemian[4], Esmaeil Akbari[5,6]*

1. School of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran
2. Pharmaceutical Sciences Research Center and Department of Toxicology and Pharmacology, School of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran
3. Department of Community Medicine, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
4. Mazandaran Heart Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
5. Department of Physiology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
6. Immunogenetics Research Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

Abstract

Introduction: Despite the experimental evidence available on the modulating effects of the H1 receptors on the autonomic function, limited research has been done the effects of such receptors through measuring the heart rate variability (HRV) indexes in human. The present study aims to assess the effect of the acute administration of different doses of hydroxyzine on the time and frequency domain of HRV indexes.

Methods: Four experiment sessions were held for the fifteen healthy participants. In each session, after the 5-minute baseline electrocardiogram (ECG) recording, one of the four interventions (the intake of a 5, 10 or 20mg hydroxyzine or a placebo) was performed and then the 5-minute ECG recordings were repeated at 30, 60, 90, 120, 180 and 240 minutes after the oral administration.

Results: The statistical analysis has shown that at minute 30, hydroxyzine has an inhibitory effect on the sympathetic index low frequency (LFnu), which is eliminated at minute 180. Moreover, from minute 60, hydroxyzine increases vagal HRV indexes, which are then eliminated at minute 240.

Conclusion: The findings of the present study showed that histamines, mediated by H1 receptors, have a modulating effect on the cardiac autonomic; however, this modulating effect is then neutralized or eliminated in a short time probably by other cardiac regulatory mechanisms.

Keywords:
Histamine;
Heart rate variability;
Hydroxyzine;
Autonomic nervous system

Received: 16 Dec 2018
Accepted: 22 Feb 2019

***Correspondence to:**
E. Akbari

Tel: +98-1144221919
Fax: +98-1144152682

Email:
akbari_esmaeil@mazums.ac.ir

Introduction

Various physiological factors are responsible for the fluctuations in successive R wave to R wave (RR) intervals of electrocardiogram (ECG), known as heart rate variability (HRV), which acts through the modulation of the final common pathways of autonomic nervous system (ANS), cardiac sympathetic and vagal branches (Stein et al., 1994; Camm et al., 1996). Two main linear approaches have been proposed for the analysis of the RR

intervals data extracted from short term ECG recordings, namely the time and frequency domain analysis (Lee et al., 2018). With each of these approaches, the calculation of certain quantities can yield an estimate of the activity pattern in cardiac sympathetic and vagal branches. Histamine is a biogenic amine that is involved in a wide range of physiological functions through four G protein-coupled receptors i.e. H1-4 (Jutel et al., 2005). Histamine H1 receptors are expressed in smooth muscle cells, myocytes and the central nervous system (Hill, 1990; Schwartz et al., 1991). Cell signaling is conducted after the activation of this receptor through the stimulation of the Gq and the production of inositol triphosphate and diacylglycerol and subsequently the increased intracellular calcium (Hill et al., 1997; Jutel et al., 2005). There are anatomical connections between the histaminergic neurons of the tuberomammillary nucleus and the regions involved in cardiac autonomic regulation, such as diagonal band of broca, paraventricular nucleus, bed nucleus of the stria terminalis and nucleus tractus solitaries (NTS) (Inagaki et al., 1988; Bealer, 1999). In addition, H1 receptors are expressed in these regions. Therefore, the H1 receptors might modulate the cardiac autonomic function. Studies have revealed that histamine through H1 receptors in central (Poole et al., 2008) and peripheral levels (Powers et al., 2001) have vagomimetic effect at the central level, by the stimulation of the dorsal nucleus of the vagus nerve (DNV) and the NTS, and at the peripheral level, by the depolarization of guinea pig cardiac ganglion. However, experimental evidence has noted the stimulating effect of histamines on the increased sympathetic activity, including the increased activity of the sympathetic neurons of the superior cervical ganglion (Snow and Weinreich, 1987; Christian et al., 1989) mediated by H1 and H3 histamine receptors (Christian and Weinreich, 1992). Despite the experimental evidence available on the modulating effects of H1 receptors on the autonomic function, little research has investigated the effects of H1 receptors on the cardiac autonomic function in human. Furthermore, these studies have reported controversial results. Some research believed that the oral administration of promethazine, which is a first-generation of H1 antihistamine, does not affect sympathovagal balance (Hattori et al., 2010), while others show that it has an augmenting effect on the cardiac vagal activity (Kavanagh et al., 2012). Hydroxyzine hydrochloride (hydroxyzine) is an H1 receptor antihistamine, a piperazine derivative and a first-generation H1 antihistamine that is easily absorbed through the gastrointestinal wall due to its lipophilic properties. As first-generation antihistamines are not G-glycoprotein substrates in the brain capillary endothelium, they are not removed from the central nervous system after crossing the blood-brain barrier and are therefore able to create central effects such as drowsiness and sedation (Matheny et al., 2001; Holgate et al., 2003; Del Cuvillo et al., 2006; Conen et al., 2013). After a single dose oral administration of hydroxyzine, the amount of time required to reach maximum plasma concentration is 2.1±0.4 hours with an elimination half-life of 20±4.1 hours (Paton and Webster, 1985; Simons, 2004; Simons and Simons, 2008). Hydroxyzine is used in the treatment of generalized anxiety disorder, chronic pruritus as well as urticaria and used acutely for pre and post operation sedation in single doses (Lader and Scotto, 1998; Del Cuvillo et al., 2006). Given H1 receptors' expression in the cardiac conduction system and in the control centers of ANS activity in the central nervous system, the effect of hydroxyzine has not been widely studied on the cardiac autonomic function. Recently, Nishiyama (2018) has investigated, as a pre-medication, the effect of intramuscular injection of hydroxyzine and midazolam on the autonomic activity of the heart in 40- to 60-year-old patients and showed that these drugs can induce transient sympathomimetic effects following intubation.

The present study therefore aims to assess the effect of the single dose oral administration of different doses of hydroxyzine on the time and frequency domain of HRV parameters. Furthermore, the findings of the present study also provide additional information to improve insights into the physiological function of H1 receptors in cardiac sympathovagal balance.

Materials and methods

Subjects

After being briefed by the researcher, 15 healthy young individuals within the age range of 21 to 25, with a body mass index (BMI) of 22.53±2.51 kg/m^2

signed the consent forms, volunteered to take part in the study. The medication used in the study was hydroxyzine and placebo tablets (made by Darou Pakhsh Holding Company, Iran). The study procedure was approved by the Ethics Committee of Mazandaran University of Medical Sciences (licensed under 91198). This study was then registered at the Iranian Registry of Clinical Trials under IRCT2014051611236N5 (http://www.irct.ir). The sample size was calculated as 15 based on data obtained from the pilot study on 5 subjects regarding the LF/HF ratio (α=0.05 and β=0.8). During the first session and before conducting the experiments, a 12-lead ECG was recorded for each subject and then examined by a cardiologist who confirmed the subjects' cardiovascular health and the absence of contraindications for hydroxyzine. The other study inclusion criteria were 'not smoking, drinking or using substances that could be abused', 'not taking medications', 'not being diagnosed with acute or chronic diseases' and 'having the normal BMI'.

Ethical approval

The volunteers provided written informed consent prior to participating. All procedures performed in this study involving human participants were in accordance with the ethical standards of the Mazandaran University of Medical Sciences (licensed under 91198) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Short-term recording of lead II of ECG

To record lead II ECG, three limb electrodes were connected to the right and left wrists and the left ankle. The volunteers were in a supine position during recording. Cardiac signals were transmitted from the electrodes to eWave ECG recorder (http://www.sciencebeam.com). This device converted cardiac signals from analogue to digital after amplification with a 1-kHz sampling rate and a 24-bit resolution. In addition to the real-time display on the computer monitor, the cardiac waves were simultaneously saved (by eProbe software) in two files in the computer memory for offline analysis: a binary ECG file and a text file containing successive RR intervals. The time series of RR intervals extracted from the 5-minute ECG recordings were eye-edited for the elimination of possible noise or ectopic heartbeats. Edited RR intervals are usually referred to as normal-to-normal (NN) intervals. The time series of NN intervals were analyzed in HRV analysis software (http://kubios.uef.fi/) as input data and the time and frequency domain indexes were measured.

Time and frequency domain indexes of short term (5-minute) HRV

The consecutive RR intervals of the electrocardiogram can be analyzed based on linear (like time and frequency domain) and non-linear methods (such as sample entropy, complexity entropy and detrended fluctuation analysis. Since the physiological interpretation of non-linear indices has not been clarified and developed well and because there are not yet reported agreed standards regarding non-linear HRV indices in the studies (Camm et al., 1996), the present study makes use of linear HRV analysis in the time and frequency domain.

An old and still valid method of quantification of the HRV is time domain analysis. The time domain measures are statistical indexes that show dispersion of data. The percentage of NN intervals with a difference of duration greater than 50ms (PNN50%) and root mean squared differences of successive NN intervals (RMSSD), which are used in short term ECG recording. In recent years, the frequency domain analysis of cardiac signals through the non-parametric method of Fast Fourier Transformation (FFT) has been widely used. FFT is a mathematical model that analyzes the time series of NN intervals into the very low frequency (VLF) band (0.003-0.04 Hz), low frequency (LF) band (0.04-0.15 Hz) and the high frequency (HF) band (0.15-0.40 Hz) and then calculates the quantitative indexes LF power and HF power.

For statistical analysis, each of the LF power and HF power measures were divided by total power spectrum minus the VLF band and reported in the normalized forms low frequency normalized unit (LFnu) and high frequency normalized unit (HFnu) (Camm et al., 1996). Another highly used index in the frequency domain analysis is the LF/HF ratio that shows cardiac sympathovagal balance.

Experiment design

In this double-blind crossover study, the subjects'

histories were noted down in their first attendance. Their 12-lead ECG was recorded and their health was confirmed by a cardiologist. The subjects were asked to return for 4 sessions of experiment with one-week intervals, given the 20-hour half-life of hydroxyzine. The subjects were advised to take a light breakfast 2 hours before the experiment and avoid caffeinated drinks and exercise the night before. All experiments were carried out in all sessions from 08:00 to 12:00 in successive stages as follows: 1) the subjects rested in the supine position for 15 minutes; 2) the 5-minute lead II ECG (baseline recording) was recorded and saved; 3) placebo or different doses of the hydroxyzine tablet (5, 10 or 20mg) were administrated and 4) the 5-minute lead II ECG at minutes 30, 60, 90, 120, 180 and 240 following administration of the medication or the placebo was recorded and saved.

Statistical analysis

The sample size calculation was based on the results of ΔLF/HF ratio obtained in a pilot study (n=5), with β=0.8 and α=0.05. This calculation suggested a sample of 15 participants in each group. Since the HRV parameter values were not normally distributed (one kolmogorov test), they were thus analyzed with nonparametric statistical methods. Friedman repeated measures analysis of variance followed by Wilcoxon tests with Bonferroni correction were used for within-group comparison of values between different time points. Kruskal-Wallis tests with post hoc multiple comparisons by Mann-Whitney U test were used to compare the values between the groups at each time points. Data was expressed as median (interquartile range). A *P* value of 0.05 or less was considered statistically significant and *P* value of less than 0.1 considered marginally significant. Data were analyzed using IBM SPSS statistics version 16.

Results

Fifteen subjects were recruited and randomized to 4 groups (G1: placebo, G2: 5mg, G3: 10mg and G4: 20mg hydroxyzine administration). Table 1 shows the median (interquartile range) values of the pre-and post-treatment HRV parameters of each group.

Report on RMSSD

RMSSD values which were significantly varied relative to baseline at various time points in G4 {χ2(df)= 29.44(4), P<0.001}, were marginally significant in G3 {χ2(df)= 11.34(4), P=0.078} and were not statistically significant in G1 and G2 (χ2(df)= 3.9(4) for G1, χ2(df)= 10.63(4) for G2, P>0.05) (Table 1). Wilcoxon tests with Bonferroni correction (effects reported at a 0.008 level of significance) revealed that these within group differences were significant only at 90min and 180min relative to baseline in G4 and 120min in G3. When RMSSD was compared in the groups, the increase in RMSSD values from baseline were not significantly different at all times after experiment { H(3)= 3.7, P=0.3 for 30min, H(3)= 2.92, P=0.41 for 60min, H(3)= 5.1, *P*=0.17 for 120min, H(3)= 1.35, *P*=0.72 for 240min} except for the marginally significant at 90min (H(3)= 7.33, P=0.06) and 180min (H(3)= 6.88, P=0.08) (Table 1). Mann Whitney tests with Bonferroni correction (effects reported at a 0.0167 level of significance) were used to follow up this finding. The increased in RMSSD values at 90min and 180min from baseline were not statistically significant among all groups and G1 (Table 1).

Report on PNN50%

PNN50% values were significantly (P=0.002) varied relative to baseline at various time points in G4 {χ2(df)= 20.83(4)} and were not statistically significant in other groups {G1(χ2(df)= 4.6(4) for G1, χ2(df)= 6.02(4) for G2, χ2(df)= 1.51(4) for G3, P>0.05) (Table 1). Wilcoxon tests with Bonferroni correction (effects reported at a 0.008 level of significance) revealed that these within group differences were significant only at 90min and 120min relative to baseline in G4. When PNN50% was compared in the groups, the increase in PNN50 values from baseline were not significantly different at all times after experiment{H(3)= 1.98, P=0.98 for 30min, H(3)= 1.07, *P*=0.78 for 60min, H(3)= 5.81, *P*=0.12 for 90min, H(3)= 6.14, *P*=0.11 for 120min, H(3)= 1.01, P=0.8 for 180min, H(3)= 0.25, P=0.97 for 240min} (Table 1).

Report on LFnu

LFnu values were significantly (P<0.05) varied relative to baseline at various time points in all the groups {χ2(df)= 14.38(4) for G2, χ2(df)= 12.83(4) for G3, χ2(df)= 17.38(4) for G4} except in G1 (χ2(df)= 1.72(4), P>0.05) (Table 1). Wilcoxon tests with

Table1: Summary of the HRV parameters analyzed from the ECG recordings of subjects, ordered by group and pre- and post-treatment periods, presented are median (interquartile range) values.

		Time						
		before	30 min	60 min	90 min	120 min	180 min	240 min
RMSSD	Placebo	37.8(29.8-71.5)	40.3(30.0-63.6)	54.4(39.0-68.6)	51.4(40.7-59.1)	56.1(33.5-67.5)	51.0(34.6-70.2)	49.7(32.3-67.3)
	5 mg	40.2(29.5-48.1)	46.5(27.0-62.2)	47.7(37.5-73.0)	51.4(40.8-67.1)	52.2(40.7-68.9)	40.5(24.6-53.2)	35.5(27.7-62.5)
	10 mg	43.3(33.4-64.6)	48.0(31.1-60.8)	51.7(33.8-66.3)	49.9(28.6-80.2)	54.3(37.6-101.5)	35.1(31.4-69.2)	42.3(32.9-53.6)
	20 mg	32.1(29.5-43.8)	46.0(32.7-65.0)	48.2(37.5-90.5)	65.0(41.0-95.0)	57.8(46.0-80.2)	58.0(35.1-68.3)	41.7(26.3-70.6)
PNN50	Placebo	28.6(9.40-41.0)	24.4(15.5-37.8)	27.0(19.3-31.4)	29.0(19.7-41.8)	36.5(12.5-42.1)	27.0(10.3-42.2)	24.4(10.5-47.1)
	5 mg	24.2(14.3-36.8)	21.2(14.2-38.7)	23.2(15.5-45.5)	21.4(16.6-48.6)	25.9(17.2-43.8)	20.2(13.7-36.4)	21.7(15.6-32.9)
	10 mg	23.7(8.90-47.4)	24.1(12.1-31.2)	25.0(13.7-45.6)	20.2(15.7-32.3)	21.7(15.9-34.2)	31.6(8.30-48.8)	25.5(14.2-53.0)
	20 mg	21.2(14.6-49.5)	25.1(12.8-55.0)	32.2(16.8-46.6)	27.5(20.2-58.6)	31.2(26.3-52.4)	34.6(13.7-50.7)	26.6(15.3-46.0)
LFnu	Placebo	53.6(41.2-76.7)	64.3(45.1-71.8)	61.3(47.8-76.0)	59.9(40.7-75.2)	57.2(48.8-67.0)	60.2(46.1-68.4)	53.9(46.0-74.4)
	5 mg	60.1(50.9-64.7)	38.7(27.6-65.2)	46.6(22.7-62.6)	36.2(23.5-56.2)	35.8(29.1-62.3)	55.6(29.2-61.6)	57.4(40.5-73.4)
	10 mg	60.2(48.5-71.4)	62.1(42.8-75.8)	40.0(27.1-57.7)	36.3(27.4-51.4)	39.9(24.9-55.3)	49.0(43.0-77.8)	64.4(46.4-77.9)
	20 mg	57.8(41.2-78.4)	54.9(29.2-68.6)	52.2(31.4-63.6)	44.8(34.2-56.9)	39.1(33.6-55.5)	55.3(33.2-76.5)	56.3(39.7-64.9)
HFnu	Placebo	54.2(48.1-73.4)	49.4(41.9-68.6)	50.5(35.8-64.0)	56.1(40.8-75.3)	56.6(46.8-65.0)	52.6(41.0-66.7)	57.0(38.8-69.0)
	5 mg	52.3(41.0-62.6)	48.5(39.5-84.6)	64.1(50.0-83.5)	57.2(45.1-82.7)	60.5(39.3-77.6)	57.8(42.3-84.2)	49.0(37.9-67.4)
	10 mg	57.2(46.7-69.6)	56.0(42.3-75.3)	75.2(52.1-91.0)	56.5(47.8-82.4)	72.9(66.3-86.1)	59.3(40.3-83.0)	51.3(39.0-71.7)
	20 mg	54.5(41.3-73.7)	61.6(51.2-82.3)	65.7(62.0-95.0)	66.9(63.2-108.4)	69.2(61.2-89.3)	68.7(51.8-77.7)	66.0(49.1-75.3)
LF/HF	Placebo	0.84(0.54-1.52)	1.30(0.66-1.71)	1.21(0.75-2.12)	1.07(0.54-1.84)	1.01(0.75-1.43)	1.14(0.76-1.75)	0.96(0.72-1.83)
	5 mg	1.20(0.70-1.47)	0.61(0.34-1.45)	0.70(0.27-1.23)	0.90(0.47-1.24)	0.70(0.40-1.25)	0.96(0.35-1.68)	1.14(0.56-1.75)
	10 mg	1.04(0.67-1.53)	1.11(0.57-1.79)	0.64(0.43-1.17)	0.53(0.30-1.27)	0.56(0.35-0.74)	0.71(0.42-1.93)	1.30(0.65-1.94)
	20 mg	0.90(0.65-1.29)	0.71(0.32-1.14)	0.98(0.34-1.09)	0.56(0.51-0.80)	0.55(0.38-0.86)	0.78(0.46-1.33)	1.09(0.54-1.56)

D: root mean squared differences of successive NN intervals; PNN50%%: percentage of NN intervals with a difference of duration greater than 50 ms; low frequency normalized unit; HFnu: high frequency normalized unit; LF/HF ratio: low frequency/high frequency ratio.

Bonferroni correction (effects reported at a 0.008 level of significance) revealed that these within group differences were significant at 60 and 90min relative to baseline in G2 and G3 groups and 30min in G2 group and 120min in G4 group. When LFnu was compared in the groups, the differences in its values from baseline were significantly different at 30, 60 and 90min after experiment {H(3)= 9.64, P=0.02 for 30min, H(3)= 11.8, P= 0.008 for 60min, H(3)=10.86, P=0.013 for 90min} and were not statistically significant at other times {H(3)= 5.92, P=0.12 for 120min, H(3)= 0.85, P=0.84 for 180min, H(3)= 1.33, P=0.72 for 240min} (Table 1). Mann Whitney tests with Bonferroni correction (effects reported at a 0.0167 level of significance) were used to follow up this finding. The decrease in LFnu values at 30min from baseline was statistically significant between G2 and G1. The decrease in LFnu values at 60min from baseline was statistically significant among all groups and G1 (Table 1).

Report on HFnu

HFnu values were significantly (P<0.05) varied relative to baseline at various time points in G3 and G4 {χ2(df)= 16.98(4) for G3, χ2(df)= 25.82(4) for G4} and were not statistically significant (P>0.05) in G1 and G2 {χ2(df)= 2.69(4) for G1, χ2(df)= 5.97(4) for G2} (Table 1). Wilcoxon tests with Bonferroni correction (effects reported at a 0.008 level of significance) revealed that these within group differences were significant only at 60min and 90min relative to baseline in G4 and 120min in G3 and G4 groups. When HFnu was compared in the groups, the differences in its values from baseline were significantly different at 60min and 120min after experiment {H(3)= 9.83, P=0.02 for 60min, H(3)= 11.1, P=0.011 for 120min}, were marginally significant at 90min after experiment {H(3)= 7.16, P=0.07} and were not statistically significant at other times {H(3)= 3.17, P=0.37 for 30min, H(3)= 1.62, P=0.66 for 180min, H(3)= 2.71, P=0.44 for 240min} (Table 1). Mann Whitney tests with Bonferroni correction (effects reported at a 0.0167 level of significance) were used to follow up this finding. The increased in HFnu values at 60min and 120min from baseline were statistically significant between G4 and G1 (Table 1).

Report on LF/HF ratio

LF/HF ratio values were significantly (P<0.001) varied relative to baseline at various time points in G3 and G4 {χ2(df)= 18.84(4) for G3 and χ2(df)= 31.77(4) for G4}, were marginally significant in G2 {χ2(df) = 12.2(4), P=0.058} and were not statistically significant (P>0.05) in G1 (χ2(df)= 3.9(4)) (Table 1). Wilcoxon tests with Bonferroni correction (effects reported at a 0.008 level of significance) revealed that these within group differences were significant only at 30min and 90min relative to baseline in G4 and 90min and 120min relative to baseline in G3. When LF/HF ratio was compared in the groups, the differences in its values from baseline were significantly different at 60min and 90min after experiment {H(3)= 10.74, P=0.013 for 60min, H(3)= 9.68, P=0.022 for 90min}, were marginally significant at 30min and 120min after experiment{P=0.08 for 30min, H(3)=6.31, P=0.098 for 120min} and were not statistically significant at other times {H(3)= 6.89, H(3)= 2.1, P=0.56 for 180min, H(3)= 0.19, P=0.98 for 240min} (Table 1). Mann Whitney tests with Bonferroni correction (effects reported at a 0.0167 level of significance) were used to follow up this finding. The decrease in LF/HF ratio values at 60min from baseline was statistically significant between all groups and G1. The decrease in LF/HF ratio values at 90min from baseline was statistically significant between G3, G4 and G1 (Table 1).

Discussion

All subjects underwent 4 sessions of experiments. At each session, after recording the 5-minute baseline ECG, one of the 4 interventions (receiving 5, 10 or 20mg doses of hydroxyzine or the placebo) was performed, and then, at minutes 30, 60, 90, 120, 180 and 240 after the oral administration of the hydroxyzine or the placebo, the 5-minute ECG recordings were repeated.

Although the purpose of the present study was not to assess the side-effects of hydroxyzine such as drowsiness, sedation or anticholinergic adverse effects, the self-reports made by subjects showed that they had feelings of drowsiness both after receiving hydroxyzine and after receiving the placebo (data not shown). Although hydroxyzine causes drowsiness and sedation as a first-generation antihistamine, the drowsiness developed in the placebo group was rather puzzling. It appears that

the subjects' 4-hour presence in the quiet environment of the laboratory and their rest in the supine position for the ECG recording have had a sleep-inducing effect. Moreover, none of the subjects complained of a dry mouth. The low doses of hydroxyzine administered in the study as well as the negligible affinity of hydroxyzine (compared to other first-generation H1 antihistamines) for muscarinic receptors might have caused the absence of anti-cholinergic effects like the dry mouth.

The present double-blind cross-over study examined the effects of 5, 10 and 20mg doses of hydroxyzine and a placebo on the activity pattern of cardiac ANS by measuring HRV indexes. Despite the various methods available for the assessment of the ANS function, this study has estimated the activity of the ANS using the time and frequency domain analyses of HRV as a simple, non-invasive and reliable method.

The findings of the present study include: 1) the placebo did not have any effects on the time and frequency domain indexes of HRV during the short term ECG recording over the 4 hours following the oral administration; 2) the 5mg hydroxyzine only reduced the LFnu at minutes 60 and 90 of the oral administration; 3) the 10 mg hydroxyzine increased the RMSSD and HFnu at minute 120 and reduced the LFnu at minutes 30, 60 and 90 and also the LF/HF ratio at minutes 90 and 120 after the oral administration, compared to the baseline values (before the administration of the medication or the placebo); 4) the 20mg hydroxyzine increased the RMSSD index at minutes 90 and 180 and also the HFnu at minutes 60, 90 and 120 following the administration of the medication, and increased PNN50% at minutes 90 and 120, and pointedly reduced LFnu at minute 120 as well as the LF/HF ratio at minutes 30 and 90 and 5) the effect of hydroxyzine on the autonomic nervous system began slightly at minute 30 of the oral administration and reached its maximum at minutes 90 and 120 and was ultimately eliminated at minutes 180 and 240.

One of the HRV indexes that showed a significant reduction with the administration of the lowest dose of hydroxyzine (5mg) was LFnu. Findings of the study showed that the 10 and 20mg hydroxyzine also reduced LFnu. Different studies have proposed contradictory biological interpretations on this index. Some believe that fluctuations in the activities of both the vagal and the sympathetic branches might be involved in determining LF (Appel et al., 1989). Some others suggest that the main factor in the development of the LF band is the sympathetic nervous system activity, especially when expressed in a normalized unit (Agelink et al., 2001).

The reduction of sympathetic activity by hydroxyzine mediated by the inhibition of histamine H1 receptors can be explained by the interaction between the histamine system and the sympathetic nervous system. Experimental findings have shown that the major part of this interaction possibly occurs at the sympathetic ganglions or at the central level. Histamine stimulates sympathetic neurons of the superior cervical ganglion (Snow and Weinreich, 1987; Christian et al., 1989) and increases the synaptic efficacy of postganglionic sympathetic neurons through H1 and H3 histamine receptors (Christian and Weinreich, 1992). Histamine is reported to activate H1 receptors directly in the membrane of preganglionic sympathetic fiber, reduce potassium conductance and induce depolarization, thereby increasing the activity of these neurons (Whyment et al., 2006).

It was found at the central level that histaminergic neurons that originate in the tuberomammillary nucleus travel to the periaqueductal gray and then project to the rostral ventromedial medulla from there (RVM) (Watanabe et al., 1984). Certain neurons travel toward the preganglionic sympathetic neurons from the RVM and stimulate the sympathetic vasomotor tone (Schreihofer et al., 2000) in addition to presenting a modulating effect on reflex control of heart rate (Alcalay et al., 1992). Seemingly, in our study the interruption in the neural circuit through H1 blockage resulting from hydroxyzine leads to inhibiting sympathetic modulation and therefore the reduced LFnu. Kavanagh et al. (2012) investigated the effect of oral administration of promethazine, a first-generation antihistamine, on autonomic HRV indexes and found those 2 hours after the oral administration, the LFnu index is reduced. Therefore, what we have found in our study i.e. the sympatholytic effect of first-generation antihistamines is consistent with the findings of their study. The present study also found that the sympatholytic effect of hydroxyzine beginning 30 minutes after its oral administration is detectable 90 and 120 minutes after and yet returns to baseline levels at minutes 180 and

240 of the administration.

Furthermore, we have observed that the different doses of hydroxyzine increase HRV indexes related to the cardiac vagal activity. Although the 5mg of hydroxyzine had no effect on vagal indexes 4 hours after its oral administration, the 10mg dose increased the time domain RMSSD and the frequency domain HFnu. Previous studies collectively claim that the two measures provide a quantity that is a good estimate of cardiac vagal activity (Camm et al., 1996). The vagomimetic or cholinergic effect of hydroxyzine was more pronounced with the 20mg doses; HFnu significantly increased at minutes 60, 90, and 120 of its oral administration and RMSSD increased at minutes 90 and 180. In addition, even PNN50%, another vagal index in the time domain analysis of HRV, increased 90 and 120 minutes.

The interesting point is that the vagomimetic effect of hydroxyzine is ultimately eliminated at minute 240. Most studies examining histamine and vagal systems' interaction have been conducted in vitro and on animal models. By whole-cell patch clamp recording of the neurons of DNV and NTS, Poole et al. (2008) showed that histamine depolarizes these neurons and that the effect is inhibited by H1 antihistamines i.e. the histaminergic pathway stimulates the dorsal nucleus vagus (DNV) through H1 receptors. The stimulation effect of histamines on vagal function is not limited to the central level; it has also been reported at the peripheral level.

Through the intracellular recording of the cholinergic neurons of guinea pig cardiac ganglion, Powers et al. (2001) showed that histamine depolarizes these neurons by stimulating H1 receptors. Seemingly, these explanations imply that hydroxyzine must inhibit cardiac vagal modulation by inhibiting H1 receptors but as it was mentioned above, hydroxyzine increases time and frequency domain indexes related to vagal function. Moreover, it is believed that first-generation H1 antihistamines exert an anticholinergic effect due to the 45% homology between H1 receptors and muscarinic receptors (Simons, 2004). Frequent evidence shows that, at low doses, anticholinergic (antimuscarinic) drugs such as atropine (Alcalay et al., 1992), scopolamine (Vybiral et al., 1990; Casadei et al., 1993), dicyclomine (Akbari and Forotan, 2009) and oxybutynin (Akbari et al., 2014) produce cholinergic effects.

The main explanation proposed for this paradoxical effect is that these compounds pass through the blood-brain barrier and stimulate vagal motor nuclei. Perhaps since hydroxyzine is able to pass through the blood-brain barrier, it has acted on muscarinic receptors of vagal motor nuclei and thereby has its cholinergic effect. This hypothesis is supported by the fact that the doses of hydroxyzine administered in the present study were sub-therapeutic doses. However, studies should be conducted in the future to confirm the mechanism of this effect. The only study to report the vagomimetic effect of first-generation H1 antihistamines in human was conducted by Kavanagh et al. (2012), who found that, in healthy young individuals, single doses of promethazine increases the frequency domain HFnu index despite; however, it had no effect on the time domain vagal indexes.

Despite the existing hydroxyzine in the plasma due to its 20-hour half-life (Simons, 2004), its impact on cardiac ANS modulation is counterbalanced after 3 hours of oral administration. This proposes that histamine H1 receptors have no significant or stable physiological role in the modulation of cardiac sympathetic and vagal activities.

The LF/HF ratio is a frequency domain index showing the sympathovagal balance. Thirty minutes after the oral administration of a 20mg hydroxyzine, the reduction of this index is not accompanied by an increase in vagal indexes. The early sympatholytic effect of hydroxyzine might have been the reason for the sympathovagal balance shift toward greater sympathetic activity at this time. Yet, at minutes 90 and 120, vagal indexes increased whereas sympathetic indexes concurrently reduced. The reduced LF/HF ratio at these times might have been due to the concurrent vagomimetic and sympatholytic effects of hydroxyzine.

In their study on the potential role of histamine systems in the incidence of autonomic nervous system dysfunction in patients with irritable bowel syndrome, Hattori et al. (2010) used a single intravenous dose of 100μg/kg of chlorphenamine (an H1 receptor antihistamine) in the 12 patients and the 12 healthy young individuals of the control group. In the healthy control group, chlorphenamine caused no change in the HF band and the LF/HF ratio, but it reduced heart rate. Although these authors attributed induced bradycardia to the inhibitory effect of

preganglionic sympathetic fibers through the inhibition of histamine H1 receptors, they did not explain the lack of change in sympathovagal balance in the healthy individuals. It appears that the increase of sympathetic activity is due to the insertion of a probe inside the rectum for creating an electrical stimulation as well as the presence of an intravenous line, probably responsible for the unchanged LF/HF ratio. In addition, they used absolute HF power (with unite of ms2) as a frequency domain index. This measure is not as accurate as HFnu (with its normalized unit) in the assessment of cardiac vagal activity (Camm et al., 1996).

Recently, Nishiyama (2018) has investigated the pretreatment effect of a combination of atropine and hydroxyzine on the cardiac autonomic activity in 40- to 60-year-old patients and concluded that sympathovagal balance trivially and transiently shifts towards sympathetic dominance following intubation; this is not consistent with what we have found. The following substantial methodological differences between our study and the Nishiyama research can justify the differences in the results: the patients vs. healthy subjects; calmness conditions vs. stressfulness conditions; male participants vs. participants from both genders; the age range between 21 and 25 vs. between 40 and 60 years of age; oral consumption vs. intramuscular injection; the duration of recording electrocardiogram in minute 40 after the drug administration vs. recording electrocardiogram from minute 5 to minute 240 after drug use as well as some other cases.

The results of the present study cannot be extended to the public, particularly to the elderly, females and clinical conditions as the subjects examined in this study consisted of healthy young men. Moreover, the results of the present study were obtained from low single doses of hydroxyzine, which may have different effects on HRV indexes and consequently on the cardiac autonomic nervous system activity in clinical conditions where it is used chronically, which could be another topic for investigation in future studies.

According to the study findings, we cannot determine the exact mechanism of hydroxyzine effect on the transient modulation of the heart autonomic function. However, considering the effect of serotoninergic and dopaminergic systems (Yeh et al., 2006; Kemp et al., 2016) on the cardiac autonomic activity and HRV parameters as well as hydroxyzine affinity to serotonin, dopamine and alpha-adrenergic receptors (Snowman and Snyder., 1990), part of hydroxyzine effects on modulation of the cardiac autonomic activity can be possibly attributed to these nonspecific effects. Surely, there is a need for future research to reveal these interactions.

Conclusion

The results of the present study showed that the stabilization of inactive histamine H1 receptors by the oral administration of single doses of sub-therapeutic hydroxyzine (5, 10 and 20mg), which can pass through the blood-brain barrier, produces symptholytic and vagomimetic effects. The symptholytic effect begins at minute 30 and is eliminated at minute 180, while the vagomimetic effect begins at minute 60 and is eliminated at minute 240. The findings of the present study show that, mediated by H1 receptors, the histamine system exerts a modulating effect on cardiac autonomic nervous system, which is then eliminated probably by other regulatory mechanisms, which is necessary to be investigated by future studies.

Acknowledgments

This work was supported by Pharmaceutical Sciences Research Center, Mazandaran University of Medical Sciences (grant number: 91198).

Conflict of interest

The authors declare no conflict of interest.

References

Agelink MW, Malessa R, Baumann B, Majewski T, Akila F, Zeit T, et al. Standardized tests of heart rate variability: normal ranges obtained from 309 healthy humans, and effects of age, gender, and heart rate. Clin Auton Res 2001; 11: 99-108.

Akbari E, Forotan SA. The effect of dicyclomine hydrochloride, an anticholinergic agent, on heart rate variability and squatting test in healthy volunteers. J Mazandaran Univ Med Sci 2009; 19: 10-7.

Akbari E, Zare AH, Alipour A. The effect of oxybutynin on cardiac autonomic modulation in healthy subjects. Clin Auton Res 2014; 24: 167-74.

Alcalay M, Izraeli S, Wallach-Kapon R, Tochner Z, Benjamini Y, Akselrod S. Paradoxical pharmacodynamic effect of atropine on

parasympathetic control: a study by spectral analysis of heart rate fluctuations. Clin Pharmacol Ther 1992; 52: 518-27.

Appel ML, Berger RD, Saul JP, Smith JM, Cohen RJ. Beat to beat variability in cardiovascular variables: noise or music?. J Am Coll Cardiol 1989; 14: 1139-48.

Bealer SL. Central neuronal histamine contributes to cardiovascular regulation. News Physiol Sci 1999; 14: 100-105.

Camm AJ, Malik M, Bigger JT, Breithardt G, Cerutti S, Cohen R, et al. Heart rate variability: standards of measurement, physiological interpretation and clinical use. Task force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Circulation 1996; 93: 1043-65.

Casadei B, Pipilis A, Sessa F, Conway J, Sleight P. Low doses of scopolamine increase cardiac vagal tone in the acute phase of myocardial infarction. Circulation 1993; 88: 353-7.

Christian EP, Undem BJ, Weinreich D. Endogenous histamine excites neurones in the guinea-pig superior cervical ganglion in vitro. J Physiol 1989; 409: 297-312.

Christian EP, Weinreich D. Presynaptic histamine H1 and H3 receptors modulate sympathetic ganglionic synaptic transmission in the guinea-pig. J Physiol 1992; 457: 407-30.

Conen S, Theunissen EL, Vermeeren A, van Ruitenbeek P, Stiers P, Mehta MA, et al. The role of P-glycoprotein in CNS antihistamine effects. Psychopharmacology (Berl) 2013; 229: 9-19.

Del Cuvillo A, Mullol J, Bartra J, Davila I, Jauregui I, Montoro J, et al. Comparative pharmacology of the H. J Investig Allergol Clin Immunol 2006; 16 Suppl 1: 3-12.

Hattori T, Watanabe S, Kano M, Kanazawa M, Fukudo S. Differential responding of autonomic function to histamine H_1 antagonism in irritable bowel syndrome. Neurogastroenterol Motil 2010; 22: 1284-91, e335.

Hill SJ. Distribution, properties, and functional characteristics of three classes of histamine receptor. Pharmacol Rev 1990; 42: 45-83.

Hill SJ, Ganellin CR, Timmerman H, Schwartz JC, Shankley NP, Young JM, et al. International Union of Pharmacology. XIII. Classification of histamine receptors. Pharmacol Rev 1997; 49: 253-78.

Holgate ST, Canonica GW, Simons FE, Taglialatela M, Tharp M, Timmerman H, et al. Consensus Group on New-Generation Antihistamines (CONGA): present status and recommendations. Clin Exp Allergy 2003; 33: 1305-24.

Inagaki N, Yamatodani A, Ando-Yamamoto M, Tohyama M, Watanabe T, Wada H. Organization of histaminergic fibers in the rat brain. J Comp Neurol 1988; 273: 283-300.

Jutel M, Blaser K, Akdis CA. Histamine in chronic allergic responses. J Invest Allergol Clin Immunol 2005; 15: 1-8.

Kavanagh JJ, Grant GD, Anoopkumar-Dukie S. Low dosage promethazine and loratadine negatively affect neuromotor function. Clin Neurophysiol 2012; 123: 780-6.

Kemp AH, Fráguas R, Brunoni AR, Bittencourt MS, Nunes MA, Dantas EM, et al. Differential associations of specific selective serotonin reuptake inhibitors with resting-state heart rate and heart rate variability: implications for health and well-being. Psychosom Med 2016; 78: 810-8.

Lader M, Scotto JC. A multicentre double-blind comparison of hydroxyzine, buspirone and placebo in patients with generalized anxiety disorder. Psychopharmacology (Berl) 1998; 139: 402-6.

Lee CH, Lee JH, Son JW, Kim U, Park JS, Lee J, et al. Normative values of short-term heart rate variability parameters in Koreans and their clinical value for the prediction of mortality. Heart Lung Circ 2018; 27: 576-587.

Matheny CJ, Lamb MW, Brouwer KR, Pollack GM. Pharmacokinetic and pharmacodynamic implications of P-glycoprotein modulation. Pharmacotherapy 2001; 21: 778-96.

Nishiyama T. Effects of premedication on heart rate variability at induction of anaesthesia: comparison between midazolam and hydroxyzine. Turk J Anaesthesiol Reanim 2018; 46: 229-232.

Paton DM, Webster DR. Clinical pharmacokinetics of H1-receptor antagonists (the antihistamines). Clin Pharmacokinet 1985; 10: 477-97.

Poole SL, Lewis DI, Deuchars SA. Histamine depolarizes neurons in the dorsal vagal complex. Neurosci Lett 2008; 432: 19-24.

Powers MJ, Peterson BA, Hardwick JC. Regulation of parasympathetic neurons by mast cells and histamine in the guinea pig heart. Auton Neurosci 2001; 87: 37-45.

Schreihofer AM, Stornetta RL, Guyenet PG. Regulation of sympathetic tone and arterial pressure by rostral ventrolateral medulla after depletion of C1 cells in rat. J Physiol 2000; 529 Pt 1: 221-36.

Schwartz JC, Arrang JM, Garbarg MO, Pollard H, Ruat M. Histaminergic transmission in the mammalian brain. Physiol Rev 1991; 71: 1-51.

Simons FE. Advances in H1-antihistamines. N Engl J Med 2004; 351: 2203-17.

Simons FE, Simons KJ. H1 antihistamines: Current status and future directions. World Allergy Organ J 2008; 1: 145-55.

Snowman AM, Snyder SH. Cetirizine: Actions on neurotransmitter receptors. J Allergy Clin Immunol 1990; 86: 1025-8.

Snow RW, Weinreich D. Presynaptic and postsynaptic effects of histamine and histamine agonists in the superior cervical ganglion of the rat. Neuropharmacology 1987; 26: 743-52.

Stein PK, Bosner MS, Kleiger RE, Conger BM. Heart rate variability: a measure of cardiac autonomic tone. Am Heart J 1994; 127: 1376-81.

Vybiral T, Bryg RJ, Maddens ME, Bhasin SS, Cronin S, Boden WE, et al. Effects of transdermal scopolamine on heart rate variability in normal subjects. Am J Cardiol 1990; 65: 604-8.

Watanabe T, Taguchi Y, Shiosaka S, Tanaka J, Kubota H,

Terano Y, et al. Distribution of the histaminergic neuron system in the central nervous system of rats; a fluorescent immunohistochemical analysis with histidine decarboxylase as a marker. Brain Res 1984; 295: 13-25.

Whyment AD, Blanks AM, Lee K, Renaud LP, Spanswick D. Histamine excites neonatal rat sympathetic preganglionic neurons in vitro via activation of H1 receptors. J Neurophysiol 2006; 95: 2492-500.

Yeh TL, Yang YK, Chiu NT, Yao WJ, Yeh SJ, Wu JS, et al. Correlation between striatal dopamine D2/D3 receptor binding and cardiovascular activity in healthy subjects. Am J Hypertens 2006; 19: 964-9.