Evaluation of Electrocardiography After Combined Use of Ondansetron and Diltiazem in Anesthetized Rats

Diltiazemin Ondansetron ile Birlikte Kullanımının Anestezi Almış Sıçan Elektrokardiyografisi Üzerindeki Etkisinin Değerlendirilmesi

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ABSTRACT Objective: This study investigated the changes in electrocardiograms in rats after combined use of ondansetron and diltiazem. **Material and Methods:** Seventeen rats were included in the study but data were collected from 11 rats with available data. After anesthesia, electrocardiogram records of rats for the initial 5 minutes were considered "Baseline" record. Following this, rats received intravenous ondansetron injection and their electrocardiograms were recorded for another 5 minutes. The same procedure was repeated with intravenous diltiazem injection. **Results:** After ondansetron injection QTc from 9 rats were longer and 2 rats were shorter compared to baseline (before the administration of ondansetron). When diltiazem was given after ondansetron, in 10 rats out of 11, the QTc time was correlated negatively with measurements obtained before diltiazem. There was no statistically significant difference between the baseline QTc measurements and the QTc obtained after the administration of diltiazem. **Conclusion:** The combined use of ondansetron and diltiazem does not lead to any change in rat electrocardiography.

Key Words: Ondansetron; diltiazem; electrocardiography

ÖZET Amaç: Bu çalışmada, sıçanlarda ondansetron ve diltiazemin birlikte kullanımının elektrokardiyografide oluşturabileceği değişikliklerin araştırılması amaçlandı. **Gereç ve Yöntemler:** Çalışmaya 17 sıçan dâhil edilmekle birlikte, veriler 11 sıçandan toplanabildi. Anesteziden sonraki ilk 5 dakika boyunca sıçanlardan elde edilen elektrokardiyogram kayıtları bazal kayıt olarak kabul edildi. Bu işlemden sonra sıçanlara intravenöz ondansetron enjekte edildi ve bunu izleyen 5 dakika boyunca elektrokardiogram kayıtları alındı. Aynı işlemler intravenöz diltiazem enjeksiyonu ile tekrar edildi. **Bulgular:** Ondansetron verildikten sonra QTc bazal değerlere (ondansetron verilmeden önce) kıyasla 9 sıçanda daha uzun ve 2 sıçanda daha kısa bulundu. Ondansetrondan sonra diltiazem verildiğinde 11 sıçanın 10'unda QTc süreleri diltizem verilmesinden önceki ölçüme göre negatif korelasyon gösterdi. Bazal elektrokardiyografi QTc değerleri ile diltiazem verilmesinden sonra elde edilen QTc değerleri arasında istatistiksel olarak anlamlı bir fark saptanmadı. **Sonuç:** Ondansetron ile diltiazemin birlikte kullanılmasının, sıçan elektrokardiografisinde değişiklik oluşturmadığı sonucuna varıldı.

Anahtar Kelimeler: Ondansetron; diltiazem; elektrokardiyografi

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Ondansetron, a 5-hydroxytryptamine-3 (5HT) receptor antagonist that is currently used to prevent postoperative nausea and vomiting, is also administered preoperatively for the same purpose.[1-3] Ondansetron also affects cardiac electrical activity leading to the development of QRS complex widening on electrocardiography (ECG), and the prolongation of QT and PR intervals.[4-6] Food and Drug Administration

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(FDA) has recently suggested that QT prolongation might result in torsade de pointes a fatal form of heart rhythm. When intravenous (IV) ondansetron is administered as a bolus, it causes stimulation of the Nucleus Tractus Solitarius, which leads to temporary bradycardia due to effects similar to baroreflex and Bezold-Jarisch Reflex responses.[7] Diltiazem, an ideal agent used in the treatment of perioperative acute hypertension, is a calcium-channel blocker, which also affects cardiac electrical activity.[8-10] Due to this effect, it causes bradycardia and a prolonged PR interval on ECG.[11,12] Since diltiazem used to treat hypertension and ondansetron used to prevent and treat nausea and vomiting cause ECG changes, coadministration of the two drugs in the same individual remains to be studied.

The goal of this study was to investigate, whether there would be any ECG changes in rat that would prevent the combined use of ondansetron and diltiazem, and thus, to question the validity of Ca^{+2} channel blocker use in combination with ondansetron.

MATERIAL AND METHODS

After the Animal Ethics Committee approved the stody protocol (IACUC approval No. 2008-62) 17 male albino Wistar rats weighing an average of 268 grams (180-344 grams) were included in the study. Rats were kept for four months at the University Experimental Research Laboratory under optimal conditions (18-21˚C). They were brought to the experiment area three days before the experiment in order to prevent possible stress on the animals, and to establish the necessary conditions for living.

Rats were given intraperitoneal 40 mg/kg of Ketamine Hydrochloride (Bayer United German Pharmaceutical Factories Co. Inc., Istanbul) and 5 mg/kg of Xylasine (Parke-Davis, Istanbul) for anesthesia. Then, a venous line was placed at the tail using a 24 G branule and ECG monitoring using a computer system was performed. Following anesthesia, a lead I electrocardiogram was recorded for five minutes as the "Baseline" record. Then 200μg/kg intravenous ondansetron was injected, and ECG was kept recording for another five minutes as the "Ondansetron" record. Finally, intravenous diltiazem 1000 μg/kg was injected and ECG recording continued for five more minutes for the same animal as the "Diltiazem" record.

All records were obtained while rats were on a ~2 millimeters thick and 45 x 65 cm metal plate connected to an amplifier ground in order to reduce noise and other interferences.

Electrocardiogram signals were amplified using a biopotential amplifier (Grass Telefactor, model P511, Grass Instrument Division, Astro-Med Inc, USA) and were recorded on a computer hard disc using a general purpose analog-digital converter card (PCL-818HG, Advantech Co, Ltd, Taiwan) at 12 bit resolution and 20 KHz sampling rate. A software program was developed by the author using the DASYLab program (V8.0, Dasytec USA, 11 Eaton Road, PO Box 748, Amherst, NH 03031-0748 USA) to verify, to record and to analyze the ECG data. To obtain adequate resolution on the ECG, the biopotential amplifier analogue filter was also set up to pass through for the frequencies less than 10 KHz. This was consistent with Nyquist frequency for the 20 KHz analogue to digital conversion and allowed the ECG signals to be examined in detail at 100 μs time resolution.

The developed software automatically cut ECG signal into segments to run the developed computer algorithm accurately. Then the segmented ECG signals were marked with dots by the algorithm automatically and then RR and QT intervals were measured accordingly (Figure 1). The software algorithms were not documented here in detail since they had some technical terminology and specific codes of the programming language. Measured data were then transferred to Microsoft Excel worksheet for some further corrections. To verify the quality of the records, each record was checked visually on computer for its distortion and consistency prior to computation. The accuracy of the algorithm was also checked for each record by randomly selecting ECG intervals and measuring the RR intervals and QT time manually, and then by comparing them to computed data on the worksheet. All were matched with each other.

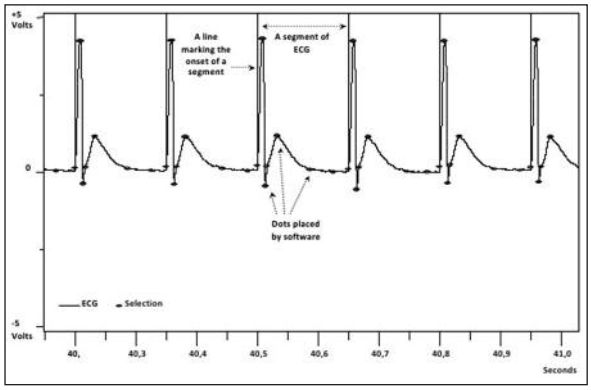

FIGURE 1: Segmented electrocardiography (ECG) record. The signal is automatically marked with dots due to signal onset, slope and sequent minimum-maximum values.

With this procedure, QT time and RR intervals were obtained for each record according to the heartbeat of the selected rats, which ranged between 654 and 1424. Then, QT time was corrected with Bazett formula and this correction was used as QTc (corrected QT) time in the evaluation. Obtained ECG data were initially analyzed and statistically tested within themselves and then with data from all experimental animals, in order to reach a conclusion.

The data were analyzed using SPSS 19.0 for Windows (SPSS, Inc., Chicago, IL). Data were presented as means ± standard deviation (SD) for numerical variables and as frequencies for categorical variables. P value less than 0.05 was considered significant. For the decision to use parametric or nonparametric methods for numeric variables, firstly normality check was done by the Shapiro-Wilks Test. Repeated measures ANOVA Test was used for the general comparison of heart rates and QTc's for three time points and after the comparison, Bonferroni Test was used to establish the statistically significant differences between the pairs.

RESULTS

Although the study included 17 rats initially, 6 rats were excluded from the analyses-5 due to vascular occlusion and one due to interference from a cell phone ringing during the experiment. From the remaining 11 rats, 33 records were obtained as "Baseline", "Ondansetron" and "Diltiazem". For each recording, at least 654 and at most 1424 measurement values were recorded based on the rats' heart rates. The comparison of "Baseline", "Ondansetron" and "Diltiazem" data revealed that ondansetron and diltiazem's QTc effects on heart rate varied significantly in opposite directions (Table 1).

Although the ECG recordings showed a significant difference after the administration of ondansetron compared to the baseline, the difference after the administration of diltiazem was not significant. In addition, no significant difference could be determined between the baseline and diltiazem QTc measurements (Table 2).

QTc from 8 rats was significantly longer than baseline after the administration of ondansetron in contrast to 2 rats with a significant decrease in QTc measurements after ondansetron (Figure 2). Compared to the results of ondansetron, after the administration of diltiazem, out of 9 rats, in 8 rats which had an increase in their QTc, a decrease was observed, while in one, an increase was observed. However in 2 rats which had a decrease, longer QTc was observed. When Diltiazem was given after Ondansetron, in 10 rats out of 11, the QTc time was observed as variable in the opposite direction (While there was an increase, it decreased and while there was a decrease it increased.) (Figure 2).

TABLE 1: Mean heart rate and mean QTc of the groups and their significance.

	Baseline	Ondansetron	Diltiazem	P Value
Heart rate	212.4 ± 68	224.9 ± 67.5	197.5 ± 52.3	0.054
QTc (miliseconds)	168.3 ± 60.9	181.1 ± 65	153.4 ± 52.2	0.016*

Values are expressed as means ± Standard Deviation.
*P<0.05 (Repeated measures ANOVA test is used to determine P value).

TABLE 2: Significance of QTc among groups.

Groups	P Value
Baseline and Ondansetron	0.029*
Ondansetron and Diltiazem	0.072
Baseline and Diltiazem	0.499

*P<0.05 (Bonferroni test is used to determine P value).

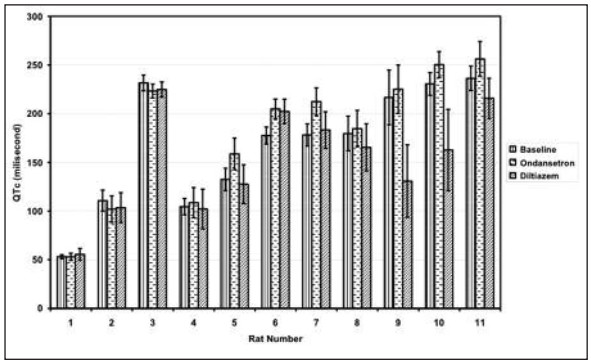

FIGURE 2: Corrected QT interval (QTc) of the rats for Baseline, Ondansetron and Diltiazem records. Error bars show standard deviation of the means.

DISCUSSION

The study may differ from other experimental research because of recording and ECG interval measurement methods. Time resolution of 100 μs in the study allowed us to achieve high precision time data to calculate QTc in comparison to conventional ECG units used in the clinics, most of which had much lower sampling rates such as 500-1000 Hz and 150-200 Hz low pass filter setting. Although conventional ECG equipment in the clinics may be an alternative to record ECG, most of them have fixed filter and sampling rate settings; thus, higher heart rate of rat might yield unreliable ECG signals.[13] On the other hand, the developed computer algorithm was also found to be successful for detecting ECG intervals. This algorithm allowed hundreds of ECG data to be automatically measured to calculate QTc. The high number of high-precision data may allow the detection of minor but statistically significant differences during any drug administration.

This study was conducted in order to evaluate the effect of IV diltiazem injected to rats that had already received IV ondansetron, on heart rate and QT interval on ECG. In contrast to the results of a study showing ondansetron administration resulting in a Bezold-Jarisch Reflex like activity leading to bradycardia,[7] in this study, an insignificant increase in heart rate was determined in rats when they were given ondansetron. Injection of diltiazem resulted in bradycardia due to the depression in the A-V node transmission;[14] but this difference was not statistically significant. The lack of a statistically significant decrease in heart rate when diltiazem was given to rats who had received ondansetron, suggests the possibility of already raised heart rates upon the previous administration of ondansetron, after which diltiazem was given. In addition, the significantly longer QTc measurements of 8 rats after ondansetron injection compared to baseline was attributed to early after-depolarization.

As also stated by Viswanathan and Rudy,[15] early after-depolarization occurs due to longer repolarization and in phase 3, the inability of K+ ions to leave the cells upon ondansetron blocking the human Ether-à-go-go-Related Gene (HERG) K1 channel,[6, 16-19] which facilitates the fast component of the potassium current (Ikr) to occur during the formation of the action potential in the heart. In our study, after the injection of diltiazem, QTc measurements reversed (increased if it was decreasing, decreased if it was increasing) in 10 out of 11 rats. This change might be associated with early after-depolarization forming due to increased intracellular positive ion state upon ondansetron administration, which is reversed by IV diltiazem preventing the entry of Ca^{+2} into the cell. In their cellular level study, Viswanathan and Rudy[15] also state that the reactivation of the L-type calcium channel current (ICa,L) prevents early after-depolarization formation. Accordingly, we believe that the QTc changes caused by ondansetron can be reversed due to ICa,L that is blocked by administering diltiazem.

In conclusion, QTc changes that occur upon ondansetron administration can be corrected by giving diltiazem and therefore, we believe that ondansetron and diltiazem used in combination can be safe.

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