American Journal of Therapeutics 19, 81–87 (2012)

Impact of Subchronic Administration of Piperazine Citrate on the Electrocardiogram of the Rat

Samuel Ghasi, PhD,* Anthony Ogbonna, MD, FMCP,
and Anthony Mbah, MD, FWACP

The effect of piperazine citrate on the electrocardiogram of the rat after treatment with piperazine citrate at 30, 60, and 100 mg/kg body weight for 16 weeks was investigated. The results were compared with a control group. There was prolongation of P-R, Q-Tc, and J-T intervals, whereas the QRS interval remained virtually unchanged. The heart rate, on the other hand, decreased in the groups that received piperazine citrate. The average heart rate in the control group was 334 ± 17.20 beats/min. In the group of rats that received the three doses of piperazine, the average heart rate at the end of a 15-minute observation period was 308 ± 3.74 beats/min, 302 ± 16.55 beats/min, and 312 ± 13.93 beats/min, respectively, and none of the values was statistically significant compared with the control. The P-R interval showed statistically significant increases in the groups treated with the three doses of piperazine over the control group. In both the 30- and 60-mg/kg groups, the average P-R interval was 92.0 ± 0.5, which was statistically significant when compared with the control average of 80.0 ± 0.00 ($P = 0.0427$). For the 100-mg/kg group, the average P-R interval was 96.0 ± 0.4 ms. The difference between this value and the control average was equally statistically significant ($P = 0.0043$). Both the Q-Tc and J-T intervals also showed statistically significant increases in the piperazine-treated groups and the P values compared with the control group were very similar. Even at the very high dose of 100 mg/kg given two times daily for 16 weeks, piperazine citrate appeared quite safe to the rat heart because it did not provoke any cardiac dysrhythmic phenomenon on the surface electrocardiogram.

Keywords: piperazine citrate, subchronic treatment, electrocardiogram, rat

INTRODUCTION

Piperazine is a useful and inexpensive anthelmintic agent active against *Ascaris lumbricoides* and *Enterobius vermicularis*.[1] Apart from its effects on the worm, piperazine has been demonstrated to have various actions on isolated tissues and organs of the mammal; it has a direct nonspecific, nonvascular smooth muscle relaxant action as it inhibits barium chloride, histamine, 5HT, and acetylcholine-induced contractions in the guinea pig ileum and rabbit duodenum by a direct smooth muscle depressant action.[2–4] It also antagonized the effect of adrenaline on the guinea pig vas deferens and oxytocin-induced contractions in the rat uterus. Also, piperazine was shown to decrease the rate and force of contraction of the isolated frog heart and the rabbit heart Langendorff preparation.[2]

We have also shown that piperazine has definite antiarrhythmic properties.[5–7] We postulated, based on our study of electrocardiographic changes induced by piperazine, that the major mechanism of the antiarrhythmic action of piperazine among others was prolongation of the cardiac action potential as a result of K^+ channels blocking activity of the drug.[5,6] These acute studies of the electrocardiographic changes induced by piperazine citrate in the conscious human subjects as well as in anaesthetized rats showed that piperazine caused dose-dependent prolongation of P-R, Q-T, and J-T intervals with little or no effects on

Department of Pharmacology and Therapeutics, College of Medicine, University of Nigeria, Enugu, Nigeria.
The authors have no conflicts of interest to declare.
Address for correspondence: Department of Pharmacology and Therapeutics, College of Medicine, University of Nigeria, Enugu 400001, Nigeria. E-mail: samuelghasi@yahoo.com

the QRS complex. In contrast, piperazine caused a dose-dependent decrease in heart rate in both studies.

In this series, we intend to investigate the electrocardiographic properties of piperazine in rats that were subchronically treated with this anthelmintic agent over a period of 4 months to elucidate the effects of piperazine on the electrocardiogram of the rat. The following parameters were measured: heart rate, PR, QRS, Q-T and J-T intervals, R-R interval, P wave, amplitude and duration and T wave amplitude. Drug-induced alterations in the electrocardiogram, after subchronic administration of piperazine in the albino Wister rat, were therefore studied.

Because an increasingly well-recognized risk of antiarrhythmic therapy is the possibility of provoking new arrhythmias, with potentially life-threatening consequences,[8] increasing concentrations of piperazine were orally given to the rats over a long period of time to determine the minimum dose level that will induce electrocardiographic changes indicative of cardiac arrhythmia/dysrhythmia.

MATERIALS AND METHODS

Forty albino Wistar rats of either sex weighing originally between 200 g and 250 g were used. The purpose of this investigation was to determine if piperazine administered to rats subchronically would induce changes in the electrocardiogram of the rat heart.

The animals were divided into four groups of 10 sex-matched rats each. Each group of rats received ad libitum one dose of piperazine, that is, 30, 60, or 100 mg/kg, given two times daily along with a standard diet. The fourth group of rats served as the control and was fed ad libitum with the standard diet only. The total period of treatment was 16 weeks.

At the end of treatment, and approximately 1 hour after last dose, the rats were anesthetized with thiopentone sodium (50 mg/kg intraperitoneally) and placed in supine position with all four limbs tied onto a dissecting board.

The animals were then connected to an electrocardiographic machine (Bioscience 400 Series Washington Oscillograph) by means of pin electrodes inserted subcutaneously into the right forelimb and left hind limb. Electrocardiographic (ECG) records were obtained on Lead II channel of the ECG machine. All ECG readings were obtained at a paper speed of 25 mm per second. ECG records were obtained at intervals of 5 minutes for a period of 15 minutes after equilibration.

Details of ECG changes were evaluated according to standard practice and included the following parameters: heart rate, P-R, R-R, QRS, Q-T and J-T intervals, R and T wave amplitudes, and the amplitude and duration of the P wave. Q-T interval corrected for heart rate (Q-Tc) was also calculated according to Onuaguluchi et al.[9]

Averages were expressed as arithmetic means ± standard error of the mean. Student *t* tests were performed comparing the results obtained from each drug concentration with measurements from control experiments and a P value of < 0.05 taken as indicating a statistically significant difference. Analysis of variance was then performed to evaluate differences between the three test groups using a one-way analysis of variance.

RESULTS

Table 1 shows the heart rate, P-R, QRS, Q-Tc and J-T intervals, P waves, and the amplitudes of T and R waves for the control hearts and hearts treated with piperazine after a 45-minute equilibration period. During the 15-minute period of observation, the value at equilibration for each parameter remained virtually unchanged.

Heart rate

A reduction in heart rate was observed in the piperazine-treated groups of rats compared with the control group after 15-minute observation.

In the control group, a peak heart rate of 380 beats/min was seen in two rats. In the same group, the least heart rate was 280 beats/min and occurred in two rats also. The average heart rate was, however, 334 ± 17.20 beats/min. In the group of rats that received 30 mg/kg piperazine, the average heart rate at the end of 15-minute observation period was 308 ± 3.74 beats/min, a 7.78% reduction compared with the control group average. The difference in the two values was statistically insignificant (P = 0.1780). Three hundred and twenty beats per minute was the peak heart rate observed in this group, whereas the least heart rate was 300 beats/min.

The group of rats administered with 60 mg/kg piperazine showed an average heart rate of 302 ± 16.55 beats/min, which was statistically not significant, compared with the control value (P = 0.2170). This was a 9.58% reduction compared with the control average heart rate.

The average heart rate observed in the group treated with 100 mg/kg piperazine was 312 ± 13.93 beats/min, which amounted to a 6.59% fall compared with the control value (P = 0.3494). The peak heart rate noted in the group was 240 beats/min and this was seen in two of the rats.

Figure 1 is a histogram showing the average heart rate in both the control rats and rats that received various doses of piperazine. Differences between the control rats and groups of rats treated with piperazine were not statistically significant.

Atrial and atrioventricular conduction

A P wave always preceded the QRS-T complex in both the control and test rats at equilibration and post-equilibration periods of observation.

Piperazine did not produce any obvious change in the amplitude and duration of the P wave. In all the animals making up the control and test groups, the duration of the P wave remained unaltered at 40 ms.

The average amplitude of the P wave was 0.11 ± 0.01 mV for the control group and groups that received piperazine at 30 and 60 mg/kg. It was, however, 0.13 ± 0.02 mV for the 100-mg/kg group. There was no statistically significant difference between the average value obtained in this group and that of the control group.

Table 1. Effect of three different doses of piperazine citrate on the electrocardiogram of the rat.

Parameter	Dose (mg/kg)	Mean ± Standard error of mean	*P* value
Heart rate (beats/min)	Control	334 ± 17.20	—
	30	308 ± 3.74	0.1780
	60	302 ± 16.55	0.2170
	100	312 ± 13.93	0.3494
			ANOVA* *P* = 0.8559
P wave (amplitude)(mV)	Control	0.11 ± 0.01	—
	30	0.11 ± 0.01	—
	60	0.11 ± 0.01	—
	100	0.13 ± 0.02	0.3972
			ANOVA *P* = 0.5314
P-R interval (seconds)	Control	0.08 ± 0.00	—
	30	0.092 ± 0.005	0.0427
	60	0.092 ± 0.005	0.0427
	100	0.096 ± 0.004	0.0043
			ANOVA *P* = 0.7828
QRS interval (seconds)	Control	0.04 ± 0.00	—
	30	0.04 ± 0.00	—
	60	0.04 ± 0.00	—
	100	0.04 ± 0.00	—
Q-Tc Interval (seconds)	Control	0.14 ± 0.006	—
	30	0.16 ± 0.00	0.0128
	60	0.17 ± 0.012	0.0460
	100	0.18 ± 0.0098	0.0150
			ANOVA *P* = 0.4451
J-T interval (seconds)	Control	0.01 ± 0.006	—
	30	0.12 ± 0.00	0.0128
	60	0.13 ± 0.012	0.0460
	100	0.14 ± 0.01	0.0150
			ANOVA *P* = 0.4451
T wave amplitude (mV)	Control	0.17 ± 0.02	—
	30	0.26 ± 0.04	0.0790
	60	0.24 ± 0.05	0.2187
	100	0.28 ± 0.03	0.0234
R wave amplitude (mV)	Control	0.45 ± 0.22	—
	30	0.61 ± 0.33	0.0039
	60	0.61 ± 0.37	0.0059
	100	0.65 ± 0.039	0.0021
			ANOVA *P* = 0.6757

*One-way analysis of variance (ANOVA) comparing the three drug-treated groups.

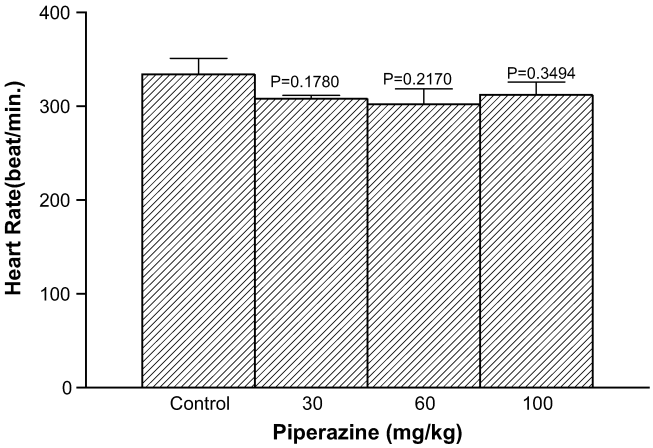

FIGURE 1. Histogram showing the average heart rate in both the control rats and rats that received 30-, 60-, and 100-mg/kg doses of piperazine. Differences between the control rats and groups of rats treated with piperazine were not statistically significant.

The P-R interval showed statistically significant increases in the groups treated with piperazine over the control group.

In the control group, a P-R interval of 80 ms was observed in all 10 rats at equilibration and during the 15-minute postequilibration period of observation. In the 30- and 60-mg/kg groups, the average P-R interval for these groups was however 92.0 ± 0.5, which was statistically significant when compared with the control average of 80.0 ± 0.00 (P = 0.0427).

Of the 10 rats that received 100 mg/kg piperazine, four had a P-R interval of 80 msec. In the remaining six rats, the P-R interval was 100 msec. However, the average P-R interval in this group of rats was 96.0 ± 0.4 ms. The difference between this value and the control average was equally statistically significant (P = 0.0043).

Figure 2 is a histogram showing the average P-R interval in respect of the control and piperazine-treated rats. The average P-R intervals of the treated groups when compared with that of the control rats were statistically significant.

Ventricular depolarization and repolarization

Piperazine did not produce any changes in the QRS interval of the Wistar rat. At the end of 4 months, the QRS interval was the same, 40 ms, in both the control rats and rats that received various doses of piperazine.

With respect to the QTc interval, piperazine caused prolongation of Q-T interval corrected for heart rate (QTc). In the control group, the average QTc interval was 0.14 ± 0.006 seconds. All 10 rats in the 30-mg/kg group had a QTc interval of 160 ms, a 14.29% increase over the average in the control group. The difference was statistically significant (P = 0.0128).

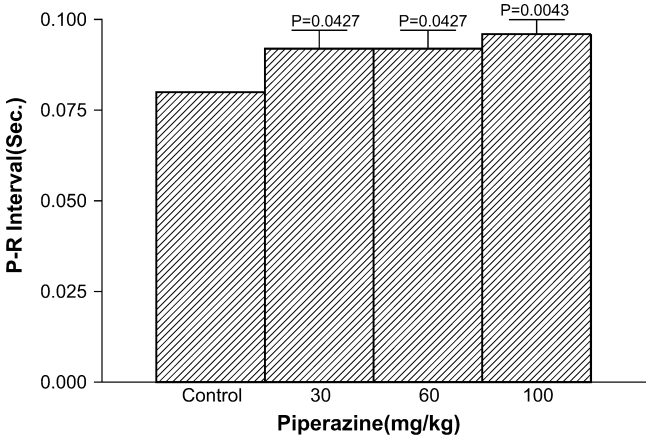

FIGURE 2. Histogram showing the average P-R interval with respect to the control and piperazine-treated rats. The average P-R intervals of the treated groups when compared with that of the control rats were statistically significant. The P values of the treated groups compared with the controls are indicated on top of the bars and showed a statistically significant difference.

Similarly, the average QTc interval for the groups that received 60 and 100 mg/kg piperazine was 0.17± 0.12 seconds (P = 0.0460) and 0.18 ± 0.001 seconds, respectively (P = 0.0150) when compared with the average QTc interval of the control group.

Figure 3 is a histogram showing the average QTc interval with respect of the control and piperazine-treated rats.

The average J-T interval for the control group of rats was 0.1 ± 0.006 seconds. Concerning the group that was subchronically treated with 30 mg/kg piperazine,

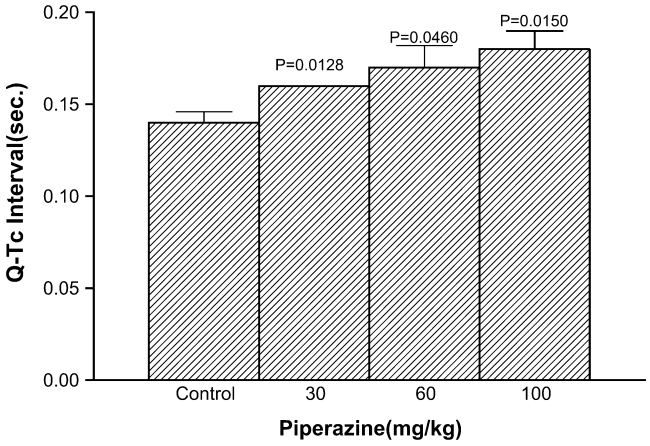

FIGURE 3. Histogram showing the average QTc interval with respect to the control and piperazine-treated rats. The P values of the means ± standard errors of mean of the piperazine-treated groups compared with the control group are given on top of the bars. The difference between the groups that received piperazine citrate and the control was statistically significant in each case.

there was a uniform J-T interval of 120 ms for all 10 rats. The average J-T interval was 0.12 ± 0.0 seconds, which is statistically significant ($P = 0.0128$) when compared with the value in control rats. In the 60-mg/kg group, the average J-T interval was 0.13 ± 0.012 seconds. The difference between this value and the control group was statistically significant ($P = 0.0460$). Four of the 10 rats that were treated with 100 mg/kg piperazine had a J-T interval of 160 ms, whereas the remaining six rats had a J-T interval of 120 ms. The average of 0.14 ± 0.01 seconds was statistically significant when compared with the value in the control rats ($P = 0.0150$).

Only the group of rats that received 100 mg/kg piperazine showed a statistically significant increase when its average T wave amplitude of 0.28 ± 0.03 mV was compared with the value of 0.17± 0.02 mV in the control group ($P = 0.0234$). In the groups treated with 30 and 60 mg/kg piperazine, the average values of the amplitudes were 0.26 ± 0.04 mV ($P = 0.0790$) and 0.24 ± 0.05 mV, respectively ($P = 0.2187$) when compared with the value in the control rats.

The average amplitude of the R wave was 0.45 ± 0.22 mV in the control group. In the 30-mg/kg group, the average amplitude was 0.61 ± 0.33 mV, a 35.6% increase over the average in the control rats. The difference between the two values was found to be statistically significant ($P = 0.0039$). Similarly, in the 60-mg/kg group, the average R wave amplitude was 0.61± 0.37 mV and statistically significant when compared with the value in the control group ($P = 0.0059$). The group that received 100 mg/kg piperazine had an average amplitude of 0.65 ± 0.03 mV. The difference between this value and the value in control rats was statistically significant ($P = 0.0021$).

Cardiac dysrhythmic phenomena

Normal (sinus) rhythm prevailed in all the 40 control and piperazine-treated rats with the P wave always followed by a QRS -T complex. However, R on T phenomenon with secondary T wave change in which the T waves appeared very prominent was seen in some of the rats that were treated with various doses of piperazine. Apart from this observation, piperazine did not induce any cardiac dysrhythmic phenomenon after subchronic oral administration in the rat.

Figure 4 shows four electrocardiograms from the control group and each of the groups treated with various doses of piperazine citrate.

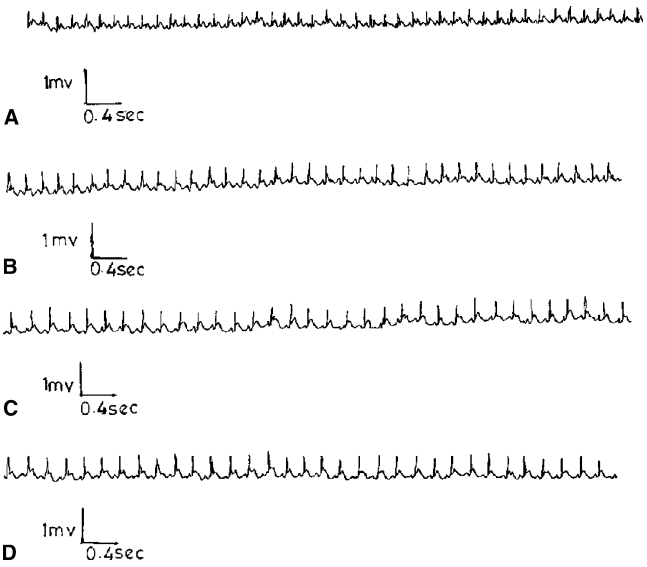

FIGURE 4. The electrocardiograms (A–D) from the control group and each of the groups treated with piperazine citrate at 30, 60, and 100 mg/kg body weight, respectively.

DISCUSSION

The results of this study showed that subchronic treatment with piperazine citrate at various doses studied produced dose-dependent electrocardiographic changes in the rat. The differences in most cases when compared with the values in controls were statistically significant. The three different doses of 30, 60, and 100 mg/kg piperazine citrate did not prove to be cardiotoxic after they were administered to the rats for 16 weeks, because there were no manifestations of ECG abnormalities at these doses at the end of the study period.

Although piperazine caused a reduction in heart rate of the rats, the decreases for all the groups were, however, erratic and not statistically significant when compared with the rats in the control group. Reduction in heart rate is typical of many antiarrhythmic drugs, especially those in Classes II to IV agents according to Vaughan-Williams classification of antiarrhythmics.[10] Some other studies of electrocardiographic effects of piperazine showed significant dose-dependent reductions in the heart rate of the rat.[6,7] An explanation for the nonstatistical difference in heart rate in this study may have to do with prolonged administration of piperazine citrate making room for homeostasis to compensate for the initial negative chronotropic effect and possibly reduced blood pressure resulting from the drug.

Piperazine citrate did not affect atrial depolarization at all the doses tested. The P wave remained virtually unchanged at 40 ms in both the control and piperazine-treated groups. There was regular sinus rhythm with every QRS complex always preceded by a positive P wave, definite evidence that the atrial musculature had been depolarized.

The three different doses of piperazine citrate used in this study prolonged the P-R interval. For all the doses, the differences compared with the control group were statistically significant. However, evaluation of differences among the three test groups using one-way analysis of variance indicates that the effect may not be dose-dependent. Prolongation of P-R interval and cardiac repolarization are usually characteristic of some typical antiarrhythmic agents.[11–14] The length of the P-R interval is a measure of the time required to depolarize atrial musculature and the current to traverse the atrioventricular nodal bundle and depolarize enough ventricular muscle to produce sufficient current to begin the QRS complex.[15] It would follow, therefore, that its prolongation in the surface ECG is an indication that piperazine causes a delay of transmission through the junctional tissue.

Most drugs that prolong action potential do so by blocking K^+ channels.[8] Increased inward Na^+ current also prolongs action potential. Because the QRS duration in this series was not altered by subchronic administration of piperazine, it is apparent that K^+ channel blockade is primarily responsible for the action potential prolongation. Therefore, antiarrhythmic property of piperazine is not principally the result of membrane-stabilizing activities, unlike some of the Class I agents.

The amplitude of the QRS complex, given by R wave deflection, was without exception significantly higher than the average R wave amplitude of the control rats. Normally, the R wave in any of the three standard leads varies between 4 and 22 mm or 0.4 and 2.2 mV.[15] Amplitudes exceeding the highest normal R wave deflection suggest the presence of cardiac disease or cardiac hypertrophy. Because R wave amplitudes in the presence of piperazine remained within the normal range, although significantly greater than in the controls, it cannot be said with certainty that some sort of cardiac disease was responsible for the high R wave amplitudes. Nevertheless, a tendency toward cardiac disease cannot be ruled out completely.

The prolongation of the Q-Tc interval of the surface electrocardiogram in the rat appeared to be related to the plasma concentration of piperazine. QT interval is a measure of ventricular action potential duration; therefore, by inference, the drug prolonged the effective refractory period. Prolongation of Q-Tc interval is attributable to slow conduction or slowed rate of repolarization. Because piperazine did not deform the QRS, prolongation of the J-T interval in the same pattern of the Q-Tc interval shows that prolongation of repolarization is a proven antiarrhythmic property of piperazine.

There was an obvious difference in the T wave amplitude between control and test groups, but it was statistically significant only in the case of the group that received 100 mg/kg piperazine compared with the control group. This may not indicate cardiac toxicity because there was no deformity of the T wave.

Several studies have reported a relationship between arrhythmia and subsequent mortality.[16–18] Other works have attributed ventricular tachycardia or ventricular fibrillation to three of every four sudden deaths.[19] Placing patients with frequent ventricular arrhythmia on prophylactic antiarrhythmic agents over time should lead to a reduction in sudden death, which in turn should lead to a reduction in total death.[17,20] It has been demonstrated that piperazine has the potential to prevent sudden cardiac death.[7] The pattern of the ECG changes caused by piperazine when given over a long period of time as seen in the present study would indicate that piperazine could be used as a prophylaxis against sudden death from ventricular tachyarrhythmias.

There is usually a large difference between effective therapeutic and overtly toxic doses of piperazine.[1] It is not, therefore, surprising to observe that high-dose piperazine (100 mg/kg) administered orally for a period of 4 months did not produce any identifiable untoward effect. Previous study showed that one must be wary of giving high-dose piperazine intravenously because of serious cardiac aberrations.[6] Therefore, in the event of using piperazine in the prophylactic management of ventricular arrhythmic conditions, giving the drug by oral route is suggested because piperazine is absorbed orally and well tolerated.

REFERENCES

1. Tracy JW, Webster LT. (2001): Drugs used in the chemotherapy of helminthiasis, In: Hardman JG, Limbird LE, Gilman AG, eds. *The Pharmacological Basis of Therapeutics*. 10th ed. New York, NY: McGraw Hill, Medical Publishing Division; 2001:1121–1140.
2. Onuaguluchi G. Some aspects of the pharmacology of piperazine citrate and the antiascaris fraction of the ethanolic extract of the bark of ERIN tree (Polyadoa umbellate-Dalziel). *West Afr Med J*. 1966;15: 22–25.
3. Onuaguluchi G. Effect of the antiascaris fraction of the ethanolic extract of the bark of Polyadoa unbellata (ERIN) and of piperazine citrate on mammalian non-vascular smooth muscle. *Pharmacologist*. 1981;23:45.
4. Onuaguluchi G. Effect of piperazine citrate and of the antiascaris fraction of the bark of Polyadoa umbellate (ERIN) on mammalian non-vascular smooth muscle. *Arch Int Pharmacodyn Therap*. 1984;269:263–270.

5. Onuaguluchi G, Ghasi S. Electrocardiographic profile of oral piperazine citrate in healthy volunteers. *Am J Ther*. 2006;13:43–47.
6. Ghasi S, Onuaguluchi G. Time course of effect of piperazine citrate on the electrocardiogram of the rat. *Am J Ther*. 2007;14:524–532.
7. Ghasi S. Piperazine protects the rat heart against sudden cardiac death from barium chloride-induced ventricular fibrillation. *Am J Ther*. 2008;15:119–125.
8. Roden DM. Antiarrhythmic drugs. In: Hardman JG, Limbird LE, Gilman AG, eds. *The Pharmacological Basis of Therapeutics*. 10th ed. New York, NY: McGraw Hill, Medical Publishing Division; 2001:933–970.
9. Onuaguluchi G, Tanz RD, McCawley E. Electrocardiographic changes induced by amrinone in the isolated perfused guinea-pig Langendorff heart preparation. *Arch Int Pharmacodyn Therap*. 1983;264:263–273.
10. Vaughan Williams EM. Classification of antiarrhythmic action reassessed after a decade of new drugs. *J Clin Pharmacol*. 1984;24:129–147.
11. Bigger JT, Hoffman BF. Antiarrhythmic drugs. In: Gilman AG, Rall TW, Nies AS, Taylor P, eds. *The Pharmacological Basis of Therapeutics*. 8th (International) ed, vol I. Oxford, England: Pergamon Press; 2002:840–870.
12. Singh BN, Nademanee K. Control of arrhythmias by selective lengthening of cardiac repolarization: theoretical considerations and clinical observations. *Am Heart J*. 1985; 109:421–430.
13. Singh BN, Sarma JSM, Zhang ZH, et al. Controlling cardiac arrhythmias by lengthening repolarization: rationale from experimental findings and clinical considerations. *Ann NY Acad Sci*. 1992;644:187–209.
14. Saksena S, Rothbart ST, Shah Y, et al. Clinical efficacy and electropharmacology of continuous intravenous amiodarone infusion and chronic oral amiodarone in refractory ventricular tachycardia. *Am J Cardiol*. 1984;54:347–352.
15. Burch GE, Winsor T. *A Primer of Electrocardiography*. 5th ed. Philadelphia: Lea & Febiger Publishers; 1969:67–145.
16. The Cardiac Arrhythmia Suppression Trial (CAST) Investigators. Preliminary report: effect of encainide and flecainide on mortality in a randomized trial of arrhythmia suppression after myocardial infarction. *N Engl J Med*. 1989;321:406–412.
17. Teo KK, Yusuf S, Furberg CD. Effects of prophylactic antiarrhythmic drug therapy in acute myocardial infarction. An overview of results from randomized controlled trials. *JAMA*. 1993;270:1589–1595.
18. Wyse DG, Waldo AL, Dimarco JP, et al. A comparison of rate control and rhythm control in patients with atrial fibrillation. *N Engl J Med*. 2002;347:1825–1833.
19. Scaffer WA, Cobb LA. Recurrent ventricular fibrillation and modes of death in survivors of out-of-hospital ventricular fibrillation. *N Engl J Med*. 1975;293:259–262.
20. Teo KK, Yusuf S. Role of magnesium in reducing mortality in acute infarction. A review of evidence. *Drugs*. 1993;46: 347–359.