Comparative study of the protective effects of terfenadine and amiodarone on barium chloride/aconitine-induced ventricular arrhythmias in rats: A potential role of terfenadine

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Abstract. Terfenadine is a second generation histamine receptor antagonist which is widely used as a non-sedating antihistamine to relieve allergic responses. However, terfenadine has been associated with a number of side effects on cardiac electrical activities through blocking multiple ion channels in the heart, particularly K^+ channels. Previous studies have also implied that terfenadine may have a potential antiarrhythmic effect; however, the electrophysiological influence by which terfenadine exerts its antiarrhythmic action remains elusive. Based on evidence from previous studies, it was hypothesized that the antiarrhythmic effect of terfenadine may be similar to that of amiodarone. The present study aimed to examine the effect of terfenadine on the QTc interval and on experimental ventricular arrhythmia in rats by comparing with that of amiodarone. The effect of terfenadine and amiodarone on the QTc interval was evaluated by comparison of multiple electrocardiograms. Barium chloride/aconitine was intraperitoneally injected to induce ventricular arrhythmias. Normal saline was administered to control rats. In comparison with normal saline, terfenadine and amiodarone similarly dose-dependently prolonged the QTc interval in rats. In the barium chloride/aconitine-induced ventricular arrhythmia model, terfenadine and amiodarone did not only similarly delay the onset time of arrhythmias induced by barium chloride (all $P<0.05$), but also increased the cumulative dosage of aconitine required to induce various arrhythmias (all $P<0.05$). Furthermore, the two drugs equivalently caused a significant decrease in the duration of ventricular tachycardia in comparison with the normal saline controls (all $P<0.05$). The present study suggested that terfenadine prolonged the QTc interval and decreased ventricular tachycardia duration. The potential protective effect of terfenadine in ventricular arrhythmia may be similar to that of amiodarone.

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Introduction

Antihistamines have been widely used to relieve a number of allergic diseases, including allergic rhinitis and conjunctivitis, chronic urticaria and histamine-induced pruritis. Terfenadine, a type of second-generation histamine H1-receptor antagonists which was discovered in the screening of antipsychotic drugs in the late 1980s, binds preferentially to peripheral rather than central H1-histamine receptors. Therefore, it exerts its antihistaminic action without impairing the individual's performance and has no effect on psychomotor skills or subjective feelings (1). However, terfenadine has been associated with a number of side effects on cardiac electrical activities. The most noticeable cardiac effect of terfenadine is the development of ventricular arrhythmias, including long QT syndrome (LQTS), torsades de pointes (TdP) and ventricular fibrillation (VF), leading to sudden mortality (2-4). Therefore, the US Food and Drug Administration decided to withdraw terfenadine from the market in the late 1990s for its severe cardiotoxicity. However, terfenadine has been reclassified as a prescription-only drug in certain countries, including the UK, Canada and China.

The cardiotoxicity of terfenadine on electrical activities was closely associated with its blockade of relevant ion channels in ventricular myocytes. The ventricular arrhythmia induced by terfenadine is mainly due to the blockade of K^+ channels (5). Blockade by terfenadine of multiple cardiac K^+ currents, particularly the fast component of delayed rectifier K^+ channel current (I_{Kr}) in different species, has been considered to account for the occurrence of TdP and the clinically observed QT prolongation (6,7). Previous studies have also found that terfenadine may potentially inhibit the Na^+ current (I_{Na}) and L-type Ca^{2+} channel current ($I_{Ca\text{-}L}$) though the blockade of the Na^+ channel and the Ca^{2+} channel, which may produce cardiotoxicity in patients with a normal heart rhythm (8). Furthermore, terfenadine inhibited $I_{Ca\text{-}L}$, I_{Na} and I_{Kr} in a potent and long-lasting manner where the currents were difficult to restore (8).

Terfenadine may have a potential antiarrhythmic effect through blocking the above ion channels, particularly K^+ channels. In a previous study, terfenadine was found to have a beneficial effect in relieving the ischemia-reperfusion injury by inhibiting the reperfusion arrhythmias in the isolated rat heart, part of which was attributable to its ability to alter the cardiac action-potential characteristics (9). A recent study also reported that the use of terfenadine alone did not produce an effect on the RR and QT intervals, QRS complex or heart rate in guinea pigs. However, a combined oral dose of terfenadine and ketoconazole significantly prolonged the RR and QT intervals and decreased the heart rate in a time-dependent manner (10). In another recent study, to detect whether the effect of terfenadine on K^+ channels was separated from the antihistaminic activity, a series of analogs of terfenadine were prepared with structural variations, and the results demonstrated that the ability to inhibit K^+ channels was generally in parallel with the antihistaminic activity (11). All of these studies suggested that terfenadine may also be used as a potential antiarrhythmic drug to a certain extent. However, the preventative and therapeutic effects of terfenadine on ventricular arrhythmias remain controversial. Furthermore, on the basis of previous studies (12,13), it was hypothesized that the antiarrhythmic effect of terfenadine may be similar to that of amiodarone, a widely used class III antiarrhythmic drug, which also exerts its antiarrhythmic effect through suppression of associated K^+ channels. The objective of the present study was to address in detail the protective and therapeutic effects of terfenadine on experimental ventricular arrhythmia in rats by comparing the antiarrhythmic activity of terfenadine with that of amiodarone, which may provide a basis for the discovery and development of novel antiarrhythmic drugs.

Materials and methods

Animals and reagents. All of the experiments were performed in accordance with the Guidelines of Animal Experiments from the Committee of Medical Ethics at the National Health Department of China (Shanghai, China) and were approved by the Laboratory Center of Shanghai Tenth People's Hospital (Shanghai, China). Sprague-Dawley rats weighing 200-250 g were purchased from the Shanghai Slac Laboratory Animal Co., Ltd (Shanghai, China), and housed in plastic cages with well-ventilated stainless steel grid tops at room temperature with a 12-h light/dark cycle. The temperature of the animal room was regulated at 23±2˚C and the relative humidity was maintained at 55±15%. All of the animals were provided free access to drinking water and normal food. Terfenadine, aconitine and dimethylsulfoxide (DMSO) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA) and amiodarone was purchased from Sanofi Pharmaceutical Co., Ltd (Paris, France). Barium chloride ($BaCl_2$) was purchased from Shanghai Chemical Reagent Research Institute Co., Ltd (Shanghai, China). The rats were anaesthetized intraperitoneally with 3% pentobarbital (30 mg/kg; China National Medicines, Co., Ltd, Shanghai, China). The standard limb lead II electrocardiogram (ECG) was measured using the BL-420S data acquisition and analysis system (Chengdu TaiMeng, Sichuan, China) following subcutaneous penetration of electrodes into four limbs. ECG intervals were expressed in milliseconds (ms) and the heart rate was expressed in beats per minute (bpm).

Measurement of ECG parameters following administration of terfenadine at different concentrations. Previous studies have reported that terfenadine prolonged the QT interval in a dose-dependent manner (10,14,15). In the present study, the impact of terfenadine on the QT interval was also examined. A total of 40 rats were randomly divided into five groups (n=8, respectively): i) Normal saline group; ii) DMSO group; iii) terfenadine 6 mg/kg group; iv) terfenadine 12 mg/kg group; v) terfenadine 18 mg/kg group. All of the drugs were administered intraperitoneally following anesthetizing the rats, and the volume of administration was 5 ml/kg. Terfenadine was dissolved in DMSO and a similar solution lacking any compound was used as a solvent control (DMSO group). ECG recordings were conducted for 90 min following drug administration, baseline ECG data were recorded for several minutes prior to administration of the compounds and continued for 90 min post-treatment. The ECG parameters, including heart rate, RR and QT intervals, were documented. The rate-corrected QT (QTc) interval was also calculated using Bazett's formula: $QTc=QT/RR^{1/2}$ (16). The QTc intervals in five groups at different time-points were compared.

Measurements of ECG parameters following administration of amiodarone. Amiodarone is an effective treatment for atrial and ventricular arrhythmias; however, its use is limited by a toxic adverse-effect profile. Amiodarone-induced K^+ channel blockade may result in prolongation of ventricular repolarization, which finally leads to LQTS, TdP and VF (17,18). As mentioned above, the impact of amiodarone on QT interval may be similar to that of terfenadine (12,13). To compare the effects of these two drugs on electrical activities, changes in the ECG caused by amiodarone treatment at different concentrations were also examined. A total of 32 rats were randomly divided into four groups (n=8/group): The normal saline (5 ml/kg), amiodarone 18 mg/kg, amiodarone 36 mg/kg and amiodarone 54 mg/kg groups. All of the drugs were administered intraperitoneally following anesthetizing the rats. The relevant ECG parameters were recorded for 90 min and compared at different time intervals.

$BaCl_2$-induced ventricular arrhythmias in rats. $BaCl_2$ is a highly toxic salt and has arrhythmogenic effects by impairing ion channels in cardiomyocytes. Multiple ventricular arrhythmias may be induced following the administration of $BaCl_2$ in rats, particularly ventricular premature contraction (VPC) and ventricular tachycardia (VT) (19,20). In the present study, the protective and therapeutic effects of terfenadine and amiodarone were detected. The experimental rats were randomly divided into the following groups: i) In the control group, 20 rats were randomly divided into two subgroups with the same treatment of 5 ml/kg normal saline as terfenadine control and amiodarone control (n=10/group); ii) in the terfenadine group, 30 rats were divided into three subgroups according to different doses of terfenadine (6, 12 and 18 mg/kg; n=10 respectively); iii) in the amiodarone group, 30 rats were divided into three subgroups according to different concentrations of amiodarone (18, 36 and 54 mg/kg; n=8 respectively). A total of 40 min following the treatment of terfenadine or amiodarone, $BaCl_2$ (4 mg/kg) was administrated via the

sublingual vein intravenously within 10 sec. The onset time of VPC, VT, VF and cardiac arrest (CA) was recorded.

Aconitine-induced ventricular arrhythmia in rats. Aconitine is well-known for its acute and high toxicity in the causation of severe arrhythmia leading to mortality (21,22). The effect of terfenadine and amiodarone on the severe arrhythmia induced by aconitine was also examined. The experimental rats were similarly divided into three groups, the subgroups and doses of terfenadine and amiodarone were exactly followed as aforementioned in the evaluation of their effects on $BaCl_2$-induced ventricular arrhythmia. A total of 40 min following the administration of terfenadine or amiodarone, aconitine (0.001%) was administered to rats via the sublingual vein in rats at a rate of 2 μg/min using an infusion pump to induce ventricular arrhythmia. The cumulative dosage of aconitine required to induce VPC, VT, VF and CA was calculated.

Measurement of the duration of VT. The impact of terfenadine and amiodarone on the duration of VT induced by $BaCl_2$ and aconitine was further examined. To reduce the incidence of aggravating VF and CA which may result in cardiac mortality, the concentrations of $BaCl_2$ and aconitine in each group were altered. A total of 40 min following treatment with terfenadine or amiodarone, $BaCl_2$ (2 mg/kg) was administered via the sublingual vein intravenously within 10 sec. Similarly, aconitine (0.001%; 20 μg/kg) was also injected via the sublingual vein intravenously within 10 sec in each group. The duration of VT was recorded and the survival rates of the different groups of animals were calculated.

Statistical analysis. Values are expressed as the mean ± standard deviation. Statistical analysis of data was performed by applying Student's t-test to determine the significance between the two groups. Statistical significance of pairwise differences among three or more groups were determined using one-way analysis of variance followed by the post-hoc test. $P<0.05$ was considered to indicate a statistically significant differnce. Analysis was performed using SPSS 16.0, (SPSS, Inc., Chicago, IL, USA). The equivalence test of terfenadine and amiodarone in shortening the duration of VT was also performed. If the 95% confidence interval (CI) of the difference between the terfenadine and amiodarone groups was within the predetermined margin of equivalence (-5 to 5), the two drugs were considered equivalent.

Results

Terfenadine prolongs the QTc interval in a dose-dependent manner. Table I details the terfenadine-induced alterations in ECG parameters of rats in five groups at different time intervals. To eliminate the impact of the heart rate on the QT interval, the effect of terfenadine on the QTc interval was detected. A total of 40 min following the administration of terfenadine at different doses, the QTc intervals in the 6, 12 and 18 mg/kg terfenadine groups were markedly increased and reached a peak (261±16, 272±3 and 280±14 ms, respectively), which demonstrated a significant difference compared with the QTc intervals immediately following terfenadine administration (238±10, 240±11 and 240±5 ms respectively,

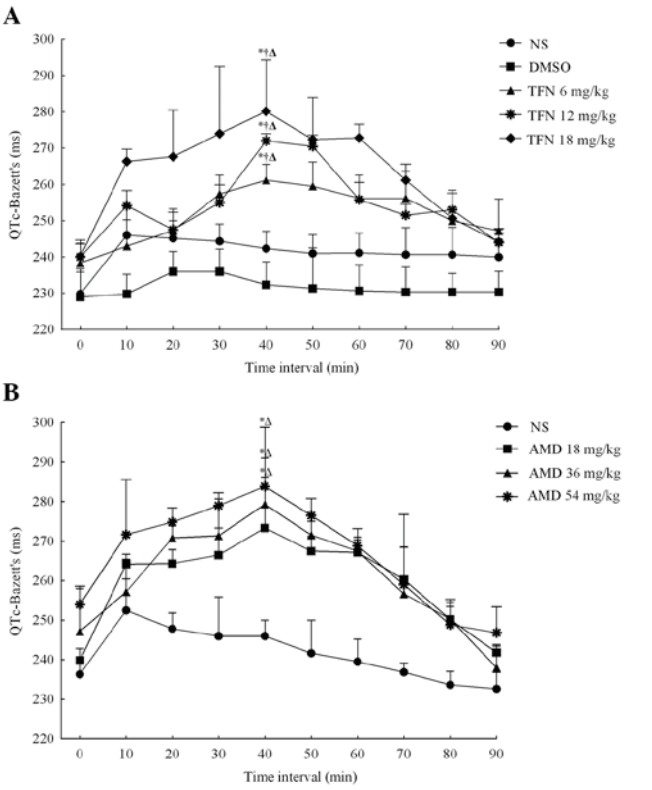

Figure 1. Changes in QTc intervals following administration of different doses of terfenadine and amiodarone to rats over different time intervals. (A) Effect of terfenadine (6, 12 and 18 mg/kg) on the QTc value at different time intervals. (B) Effect of amiodarone (18, 36, and 54 mg/kg) on the QTc value at different time intervals. Terfenadine and amiodarone prolonged the QTc intervals to a similar extent in a dose-dependent manner, and the QTc intervals reached a peak 40 min following injection of terfenadine and amiodarone. In each group (n=8), values are expressed as the mean ± SD. $^{*}P<0.05$ versus value at time interval=0; $^{\dagger}P<0.05$ versus DMSO group; $^{\Delta}P<0.05$ versus NS group. NS, normal saline; DMSO, dimethylsulfoxide; TFN, terfenadine; AMD, amiodarone.

all $P<0.05$; Fig. 1A). Following this, the QTc intervals gradually declined in these groups and finally restored to baseline in 90 min. The QTc intervals demonstrated no significant alterations in the normal saline and DMSO groups following administration and no statistical difference was detected between these two groups. However, in the presence of the selected doses of terfenadine, the ECG demonstrated that the QTc intervals increased in a dose-dependent manner, as demonstrated in Fig. 1A. Furthermore, terfenadine may also dose-independently alter RR and QT intervals as well as the heart rate. All of these results were consistent with the aforementioned previous studies (14,15), which suggested terfenadine prolonged the QT interval in a dose-dependent manner.

Amiodarone exerts similar effects on the QTc interval to that of terfenadine. By contrast, the effects of amiodarone on the QTc intervals of rats were further assessed and the data revealed that amiodarone had a similar effect on the ECG parameters to that of terfenadine. The results are outlined in Table II. Intraperitoneal injection of amiodarone also resulted in dose-dependent QTc interval prolongation, as is demonstrated in Fig. 1B. A total of 40 min following administration, the

Table I. Terfenadine-induced alterations of RR (ms), HR (bpm), QT (ms) and QTc-Bazett's (ms) at different time intervals.

	Time interval (min)									
Parameters/groups	0	10	20	30	40	50	60	70	80	90
RR (ms)										
NS	176±8	185±6	184±7	185±6	184±5	182±11	182±5	185±7	183±9	182±12
DMSO	175±11	177±9	179±12	179±7	177±11	177±21	176±10	176±14	175±11	174±14
TFN 6 mg/kg	180±17	185±14	186±4	191±1	200±17[a,b,c]	200±34	188±31	187±2	186±5	185±10
TFN 12 mg/kg	183±11	189±7	185±7	190±12	207±23[a,b,c]	205±49	205±32	187±23	188±22	186±9
TFN 18 mg/kg	187±12	199±6	202±31	207±23	214±28[a,b,c]	213±25	208±9	202±8	189±23	185±12
HR (bpm)										
NS	342±16	326±11	325±13	326±11	327±8	330±20	330±9	325±11	330±19	330±15
DMSO	345±21	341±18	337±22	336±13	339±21	342±12	343±21	343±27	344±22	345±14
TFN 6 mg/kg	336±31	326±23	323±7	314±2	302±24[a,b,c]	308±27	328±30	322±3	323±14	327±9
TFN 12 mg/kg	328±22	319±12	325±11	316±20	294±14[a,b,c]	308±19	208±49	325±42	328±17	328±6
TFN 18 mg/kg	322±20	303±11	303±40	293±33	284±26[a,b,c]	285±20	288±11	298±13	320±18	326±23
QT (ms)										
NS	96.4±3.3	106±4	105±3	105±3	104±2	103±4	103±2	103±4	103±4	102±9
DMSO	95.6±4.1	96.5±3.2	99.7±9.9	99.7±1	97.9±5.3	97.2±9.6	96.6±3.4	96.4±1.7	96.7±2.6	96.1±4.9
TFN 6 mg/kg	101±3	104±4	106±2	113±2	117±7[a,b,c]	116±10	110±10	111±3	105±8	104±13
TFN 12 mg/kg	103±3	110±2	106±3	111±5	123±8[a,b,c]	122±16	115±15	109±6	105±6	104±21
TFN 18 mg/kg	104±4	106±3	120±10	125±8	129±8[a,b,c]	126±8	125±3	117±2	106±15	103±11
QTc-Bazett's (ms)										
NS	230±11	246±10	245±5	244±9	242±15	241±5	241±15	241±7	241±8	240±8
DMSO	229±8	230±5	237±15	236±6	232±6	231±13	231±7	230±7	230±5	230±6
TFN 6 mg/kg	238±10	243±7	247±6	257±15	261±16[a,b,c]	260±7	254±6	254±7	250±8	247±9
TFN 12 mg/kg	240±11	254±4	248±5	255±9	272±3[a,b,c]	271±3	256±5	252±13	253±5	244±3
TFN 18 mg/kg	240±5	266±4	268±14	274±19	280±14[a,b,c]	274±12	273±4	261±4	251±3	244±4

In each group (n=8), values are presented as the mean ± standard deviation. [a]P<0.05 vs. value at time interval=0; [b]P<0.05 vs. the DMSO group; [c]P<0.05 vs. the NS group. NS, normal saline; DMSO, dimethylsulfoxide; TFN, terfenadine; HR. heart rate; bpm, beats per minute.

QTc intervals in the different groups all reached a peak. The QTc interval was 240±3 ms, 247±10 ms and 254±5 ms immediately following amidarone administration and increased to 272±17, 279±14 and 284±14 ms following 40 min in 18, 36 and 54 mg/kg amiodarone groups, respectively (all P<0.05), which demonstrated a significant difference when compared with the normal saline group 40 min following amidarone administration (246±4 ms) in these groups (all P<0.05). The data demonstrated that the mechanism underlying the prolongation of QTc intervals induced by terfenadine and amiodarone was similar, since terfenadine as well as amiodarone are blockers of the K^+ channel encoded by the human ether-à-go-go-related gene (hERG) (23,24).

Effects of terfenadine and amiodarone on $BaCl_2$-induced ventricular arrhythmia in rats. Intravenous injection of $BaCl_2$ into rats produced disturbances of the cardiac rhythm, including VPC, VT, VF or CA (Fig. 2A). Generally, VPC occurred first and was followed by aggravating VT and VF. Eventually, several animals died as a result of VF or CA. VPC and VT appeared in all of the animals following $BaCl_2$ treatment. The incidence of VF or CA varied in the different groups and was possibly reduced by terfenadine and amiodarone. As revealed in Fig. 2B, only six, three and four animals exhibited VF in the 6, 12 and 18 mg/kg terfenadine groups as opposed to animals in the terfenadine control group. VF was triggered in seven, three and three animals in the 18, 36 and 54 mg/kg amiodarone groups, respectively. Compared with nine animals in the amiodarone control group, the incidence of VF was significantly reduced by amiodarone (Fig. 2C). Likewise, the CA incidence declined with varying degrees following treatment with terfenadine and amiodarone at different doses.

Intravenous administration of 18, 36 and 54 mg/kg amiodarone significantly delayed the onset time of VPC, VT, VF and CA (all P<0.05 vs. amiodarone control group). In a similar manner, 12 and 18 mg/kg terfenadine also markedly delayed the onset time of VPC, VT, VF and CA (all P<0.05 vs. the terfenadine control group; Table III and Fig. 3). However, it should be noted that 6 mg/kg

Table II. Amiodarone-induced alterations of RR (ms), HR (bpm), QT (ms) and QTc-Bazett's (ms) at different time intervals.

	Time interval (min)									
Parameters/Groups	0	10	20	30	40	50	60	70	80	90
RR (ms)										
NS	179±24	191±14	187±13	185±19	185±22	185±19	182±12	180±19	177±14	177±21
AMD 18 mg/kg	184±20	201±12	201±16	202±18	203±15[a,b]	201±26	201±13	200±13	191±9	183±11
AMD 36 mg/kg	185±5	191±28	205±25	206±18	218±16[a,b]	203±27	202±18	193±12	191±5	179±5
AMD 54 mg/kg	192±26	208±20	214±18	216±6	222±7[a,b]	214±6	202±24	200±17	191±9	186±6
HR (bpm)										
NS	341±28	316±22	322±23	332±27	333±11	328±36	332±22	337±36	343±12	342±18
AMD 18 mg/kg	329±37	305±11	301±25	299±18	297±23[a,b]	303±11	308±15	302±20	314±31	328±24
AMD 36 mg/kg	325±8	320±20	296±17	294±15	276±21[a,b]	300±25	306±32	311±18	317±15	339±17
AMD 54 mg/kg	317±45	290±28	283±24	278±7	271±9[a,b]	281±8	310±35	302±12	316±5	328±13
QT (ms)										
NS	99.6±6.1	110±10	107±4	106±12	105±8	104±10	102±3	100±5	100±9	99.8±5.7
AMD 18 mg/kg	103±7	118±9	118±5	120±6	122±6[a,b]	120±9	119±12	116±9	112±5	109±8
AMD 36 mg/kg	106±14	112±8	123±7	123±6	130±10[a,b]	122±8	120±12	113±13	111±7	102±15
AMD 54 mg/kg	111±8	124±7	127±16	123±2	134±17[a,b]	128±2	120±12	116±19	111±5	106±12
QTc-Bazett's (ms)										
NS	236±3	253±14	248±4	246±11	246±4	242±9	240±6	237±2	234±3	233±4
AMD 18 mg/kg	240±3	264±3	264±4	267±16	272±17[a,b]	268±4	267±3	260±9	250±3	242±2
AMD 36 mg/kg	247±10	257±3	271±3	271±2	279±14[a,b]	272±4	268±4	257±12	250±4	238±6
AMD 54 mg/kg	254±5	272±4	275±11	279±3	284±14[a,b]	277±4	269±4	259±18	249±6	247±7

In each group (n=8), values are presented as the mean ± standard deviation. [a]P<0.05 vs. 0-time interval; [b]P<0.05 vs. the NS group. NS, normal saline; AMD, amiodarone; HR. heart rate; bpm, beats per minute.

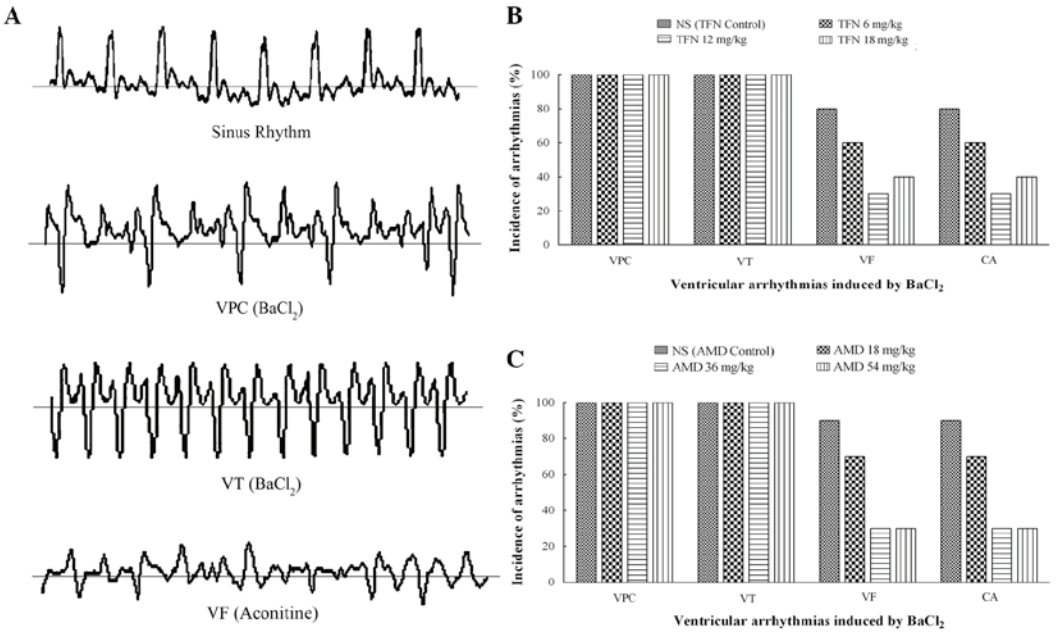

Figure 2. Multiple ventricular arrhythmias induced by $BaCl_2$ or aconitine. (A) Representative electrocardiogram recordings with sinus rhythm in a healthy rat and with ventricular arrhythmias in a rat treated with $BaCl_2$ or aconitine. (B) Incidence of multiple ventricular arrhythmias induced by $BaCl_2$ in each group. In all of the animals, VPC and VT were triggered successfully. The incidence of VT and CA was significantly reduced following administration of terfenadine and amiodarone. In each group, n=10. VPC, ventricular premature contraction; VT, ventricular tachycardia; VF, ventricular fibrillation; CA, cardiac arrest; NS, normal saline; TFN, terfenadine; AMD, amiodarone.

Table III. Onset time of $BaCl_2$-induced ventricular arrhythmias in different groups.

Groups	Onset time of VPC (s)	Onset time of VT (s)	Onset time of VF (s)	Onset time of CA (s)
Terfenadine				
NS (TFN control)	6.89±1.37	10.02±1.35	14.68±1.05	45.07±7.03
TFN 6 mg/kg	6.96±1.70	12.05±1.33[b]	19.10±4.17[b]	70.82±8.98[b]
TFN 12 mg/kg	8.66±1.56[a]	15.35±1.94[b]	23.44±2.47[b]	79.93±7.15[b]
TFN 18 mg/kg	9.24±1.51[b]	20.23±1.25[b]	32.07±3.62[b]	87.90±5.91[b]
Amiodarone				
NS (AMD control)	6.83±1.55	10.19±0.84	14.58±1.77	48.67±7.42
AMD 18 mg/kg	8.17±1.63	12.50±2.24[d]	20.82±1.76[d]	74.62±7.57[d]
AMD 36 mg/kg	8.75±1.71[c]	15.27±1.20[d]	24.12±2.01[d]	81.57±7.41[d]
AMD 54mg/kg	9.48±1.69[d]	20.46±1.63[d]	34.97±1.31[d]	86.13±11.24[d]

In each group (n=10), values are presented as the mean ± standard deviation. [a]P<0.05 and [b]P<0.01 vs. the NS (TFN Control) group; [c]P<0.05 and [d]P<0.01 vs. the NS (AMD Control) group. VPC, ventricular premature contraction; VT, ventricular tachycardia; VF, ventricular fibrillation; CA, cardiac arrest; NS, normal saline; TFN, terfenadine; AMD, amiodarone.

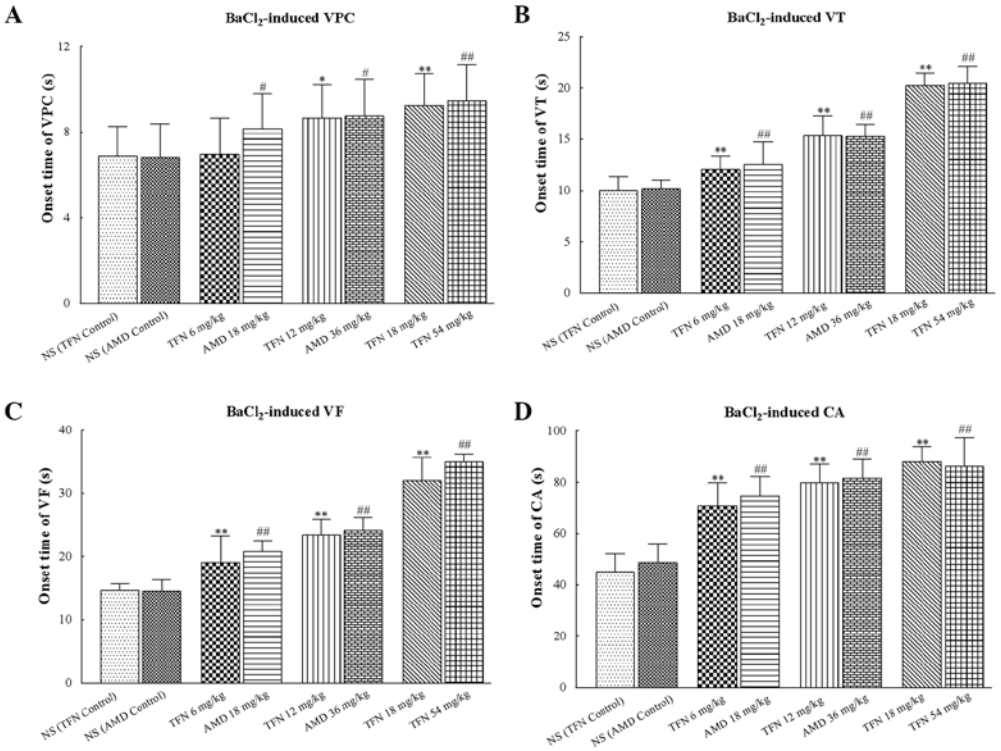

Figure 3. Onset time of $BaCl_2$-induced ventricular arrhythmias in each group. (A, B, C and D) The onset time of VPC, VT, VF and CA induced by $BaCl_2$, respectively. Low dose (6 mg/kg) terfenadine may not delay the onset time of VPC but delayed the onset time of VT, VF and CA. AMD of different concentrations significantly delayed the onset time of VPC, VT, VF and CA. In addition, 12 and 18 mg/kg terfenadine also markedly delayed the onset time of VPC, VT, VF and CA. In each group (n=10), values are expressed as the mean ± SD (columns, mean; error bars, ± SD) *P<0.05 and **P<0.01 vs the NS (TFN control) group; $^{\#}$P<0.05 and $^{\#\#}$P<0.01 vs. the NS (AMD control) group. VPC, ventricular premature contraction; VT, ventricular tachycardia; VF, ventricular fibrillation; CA, cardiac arrest; NS, normal saline; TFN, terfenadine; AMD, amiodarone; SD, standard deviation.

terfenadine was not able to delay the onset time of VPC (P=0.07 vs. the terfenadine control group), but was able to delay the onset time of VT, VF and CA (all P<0.05 vs. the terfenadine control group).

Effects of terfenadine and amiodarone on aconitine-induced ventricular arrhythmia in rats. Ventricular premature beats were followed by VT and VF appearing in all treated rats following administration of aconitine (Fig. 2A). Treatment of the rats with terfenadine or amiodarone prior to aconitine caused a significant increase in the cumulative dosage of aconitine required to induce VPC, VT, VF and CA compared with the terfenadine control and amiodarone control groups, respectively (P<0.05 or P<0.01; Table IV and Fig. 4).

Table IV. Dosage of aconitine required to induce ventricular arrhythmias in different groups.

Groups	Dosage of aconitine (μg/kg)			
	VPC	VT	VF	CA
Terfenadine				
NS (TFN control)	11.52±1.43	15.17±1.99	18.70±1.04	82.65±7.73
TFN 6 mg/kg	14.34±1.30[b]	16.75±0.98[a]	21.32±1.29[b]	92.08±11.59[a]
TFN 12 mg/kg	18.54±1.73[b]	20.45±1.76[b]	33.97±3.11[b]	116.61±25.29[b]
TFN 18 mg/kg	20.95±1.35[b]	22.28±1.83[b]	40.19±1.61[b]	155.83±24.14[b]
Amiodarone				
NS (AMD control)	11.36±1.19	15.83±2.26	18.75±1.68	83.41±5.98
AMD 18 mg/kg	17.17±1.90[d]	20.27±2.32[d]	23.68±1.47[d]	94.81±16.88[c]
AMD 36 mg/kg	20.04±1.22[d]	22.90±1.74[d]	36.85±2.18[d]	120.29±33.05[d]
AMD 54 mg/kg	22.20±2.20[d]	24.74±2.98[d]	41.27±2.01[d]	164.21±13.41[d]

In each group (n=10), values are presented as the mean ± standard deviation. VPC, ventricular premature contraction; VT, ventricular tachycardia; VF, ventricular fibrillation; CA, cardiac arrest; NS, normal saline; TFN, terfenadine; AMD, amiodarone. [a]P<0.05 and [b]P<0.01 versus NS (TFN control) group; [c]P<0.05 and [d]P<0.01 versus NS (AMD control) group.

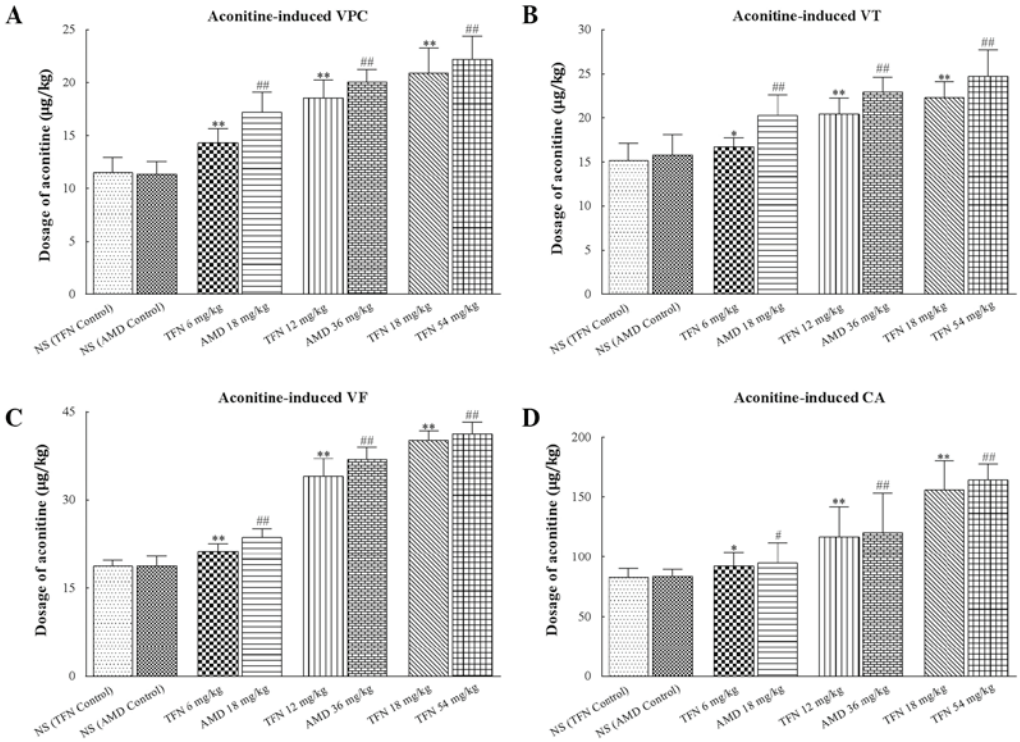

Figure 4. Dosage of aconitine required to induce ventricular arrhythmias in each group. (A-D) The cumulative dosage of aconitine required to induce VPC, VT, VF and CA, respectively. Treatment of rats with terfenadine and amiodarone prior to aconitine both caused significantly increase in the cumulative dosage of aconitine required to induce VPC, VT, VF and CA. In each group (n=10) values are presented as the mean ± SD (columns, mean; error bars, ± SD). [#]P<0.05 and [##]P<0.01 versus NS (AMD control) group. VPC, ventricular premature contraction; VT, ventricular tachycardia; VF, ventricular fibrillation; CA, cardiac arrest; NS, normal saline; TFN, terfenadine; AMD, amiodarone. [*]P<0.05 and [**]P<0.01 versus NS (TFN control) group.

Effect of terfenadine and amiodarone on the duration of VT in rats. As demonstrated in Table V and Fig. 5, treatment of rats with 12 and 18 mg/kg terfenadine prior to $BaCl_2$ or aconitine significantly shortened the duration of VT (P<0.05 or P<0.01). Similarly, the duration of $BaCl_2$/aconitine-induced VT in 18, 36 and 54 mg/kg amiodarone groups was markedly reduced compared with that of the amiodarone control group. However, 6 mg/kg terfenadine exerted no essential impact on the duration of VT induced by $BaCl_2$ and aconitine (P=0.06 and P=0.09 vs. the terfenadine control group, respectively). Furthermore, according to the results of the equivalence test of 12 mg/kg terfenadine and 36 mg/kg amiodarone, 18 mg/kg terfenadine and

Table V. Duration of $BaCl_2$/aconitine-induced VT in different groups.

Groups	Duration of $BaCl_2$-induced VT (min)	Duration of aconitine-induced VT (min)
Terfenadine		
NS (TFN control)	29.17±4.32	53.27±7.20
TFN 6 mg/kg	29.03±2.13	54.03±6.94
TFN 12 mg/kg	19.15±3.15[a]	38.89±5.22[b]
TFN 18 mg/kg	12.88±1.84[b]	24.93±5.04[b]
Amiodarone		
NS (AMD control)	30.83±4.88	52.71±9.62
AMD 18 mg/kg	24.69±2.37[c]	46.33±5.06[c]
AMD 36 mg/kg	19.69±3.75[d]	39.02±4.76[d]
AMD 54 mg /kg	11.55±1.73[d]	25.12±4.23[d]

In each group (n=10) values are presented as the mean ± SD. [a]P<0.05 and [b]P<0.01 vs. the NS (TFN control) group; [c]P<0.05 and [d]P<0.01 vs. the NS (AMD control) group. VT, ventricular tachycardia; NS, normal saline; TFN, terfenadine; AMD, amiodarone.

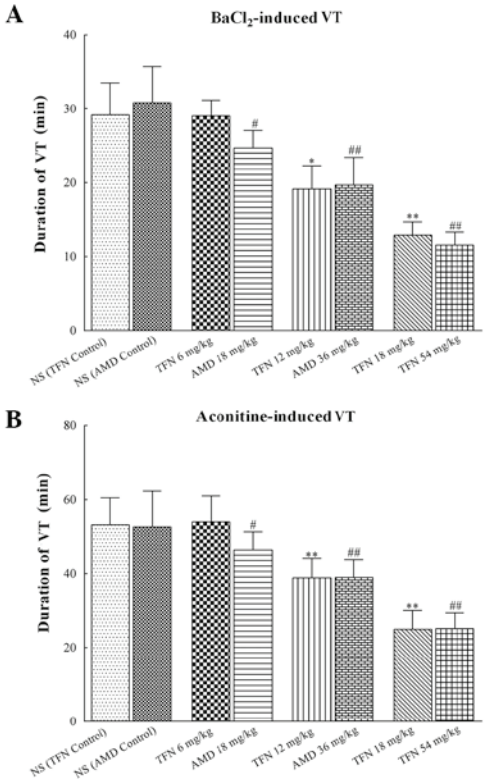

Figure 5. Duration of $BaCl_2$/aconitine-induced VT in each group. (A) Duration of $BaCl_2$-induced VT in different groups. (B) Duration of aconitine-induced VT in different groups. Administration of 12 and 18 mg/kg terfenadine significantly shortened the duration of VT. Similarly, the duration of $BaCl_2$/aconitine-induced VT in 18, 36 and 54 mg/kg amiodarone groups were markedly shortened. In each group (n=10) values are expressed as the mean ±SD (columns, mean; error bars, ± SD). *P<0.05 and **P<0.01 vs. the NS (TFN control) group; #P<0.05 and ##P<0.01 vs. the NS (AMD control) group. VT, ventricular tachycardia; $BaCl_2$, barium chloride; NS, normal saline; TFN, terfenadine; AMD, amiodarone; SD, standard deviation.

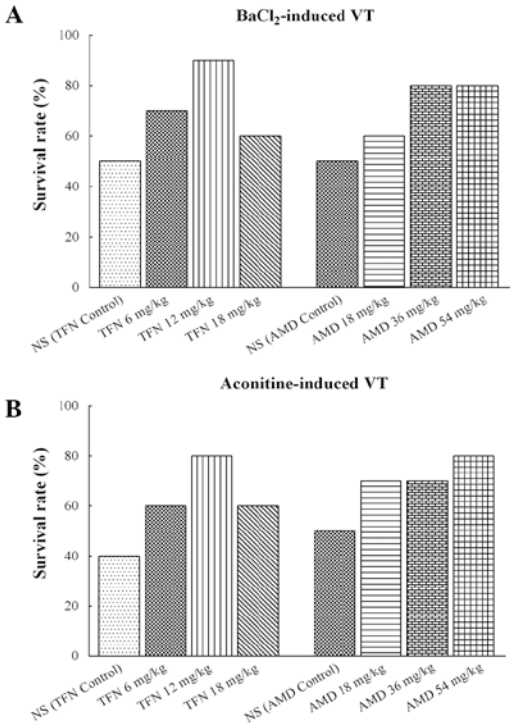

Figure 6. Survival rates in different groups following triggering of VT with $BaCl_2$ or aconitine in rats. (A) Survival rates of terfenadine- and amiodarone-treated rats with $BaCl_2$-induced ventricular arrhythmia. The survival rate in the normal control group was 50%, in the 18 mg/kg terfenadine and 18 mg/kg amiodarone groups it was 60%, in the 6 mg/kg terfenadine group it was 70%, in the 36 and 54 mg/kg amiodarone groups it was 80%, and in the 12 mg/kg terfenadine it was 90%. (B) Survival rates of terfenadine and amiodarone on aconitine-induced ventricular arrhythmia. The survival rate in the terfenadine control group was 40%, in the amiodarone control group it was 50%, in the 6 and 18 mg/kg terfenadine groups it was 60%, in the 18 and 36 mg/kg amiodarone groups it was 70%, and in the 12 mg/kg terfenadine and 54 mg/kg amiodarone groups it was 80%. Each group, n=10. VT, ventricular tachycardia; NS, normal saline; TFN, terfenadine; AMD, amiodarone.

54 mg/kg amiodarone in reducing the duration of VT caused by $BaCl_2$, the 95% CI of the difference (-3.6-2.4) and (-0.2-2.9) were both within the predetermined margin of equivalence. Similarly, regarding the aspect of suppressing aconitine-induced VT, the 95% CI (-4.5-4.2) and (-4.3-3.9) also lay within the predetermined margin of equivalence. These results indicated that the potential antiarrhythmic effect of terfenadine was not inferior to that of amiodarone.

Administration of $BaCl_2$ caused life-threatening VT in several animals in all of the groups. The incidence of mortality reached 50% (5/10 animals) in the terfenadine control and amiodarone control groups, 40% (4/10 animals) in the 18 mg/kg terfenadine and 18 mg/kg amiodarone groups, 30% (3/10 animals) in the 6 mg/kg terfenadine group, 20% (2/10 animals) in the 36 and 54 mg/kg amiodarone groups, and 10% (1/10 animals) in the 12 mg/kg terfenadine group (Fig. 6A). Aconitine also caused life-threatening VT in each group. The incidence of mortality reached 60% (6/10 animals) in the terfenadine control group, 50% (5/10 animals) in the amiodarone control group, 40% (4/10 animals) in the 6 and 18 mg/kg terfenadine groups, 30% (3 /10 animals) in the 18 and 36 mg/kg amiodarone groups, and 20% (2/10 animals) in the 54 mg/kg amiodarone group (Fig. 6B).

Discussion

In the present study, $BaCl_2$/aconitine-induced animal models of ventricular arrhythmia were established to investigate and compare the protective effects of terfenadine and amiodarone at different concentrations on ventricular arrhythmia. The results demonstrated that both terfenadine and amiodarone significantly prolonged the QTc intervals and altered the other ECG parameters in a dose-dependent manner. In the meantime, these two drugs demonstrated similar preventative and therapeutic effects on various arrhythmias triggered by $BaCl_2$ or aconitine.

It is well established that infusion of $BaCl_2$ leads to delayed afterdepolarization and triggered activity through increases in Na^+ and Ca^{2+} inflow to the myocardium, promoting the genesis of arrhythmia (25,26). Most importantly, electrophysiological studies have found that Ba^{2+} was the potential blocker of various K^+ channels and permeation of Ba^{2+} through the K^+ channel may impede the presence of K^+ in the external solution, which may result in the increase of autorhythmicity of the myocardium to produce ventricular arrhythmia (27-29). The present study demonstrated that terfenadine and amiodarone at different concentrations not only delayed the onset time of VPC, VT, VF and CA in $BaCl_2$-induced ventricular arrhythmia in rats, but terfenadine and amiodarone at different doses also shortened the duration of VT. Additionally, the survival rates in all of the terfenadine and amiodarone groups were higher than those of the control groups. The above results may be mainly explained by K^+ channel blockade of terfenadine and amiodarone, and partially attributed to the Na^+ and Ca^{2+} channel blockade of these two drugs (30,31).

Aconitine, a specific Na^+ channel blocker able to prolong the open state of the channel, may induce intracellular Na^+ accumulation and intracellular Ca^{2+} overload, which may eventually result in polymorphic ventricular arrhythmia (32). The present study indicated that there was an obvious protective effect of the different concentrations of terfenadine and amiodarone on aconitine-induced arrhythmia. Terfenadine and amiodarone of different doses significantly increased the threshold dose of aconitine required to induce ventricular arrhythmias, including VPC, VT, VF and CA. Furthermore, the duration of ventricular arrhythmias and the mortality were significantly reduced compared with those in the control groups. The protective effect may be due to decrease of I_{Na} and $I_{Ca\text{-}L}$ currents induced by these two drugs in ventricular cardiomyocytes (4,33,34).

However, low-dose terfenadine (6 mg/kg) exerted no significant impact on VPC and the duration of ventricular tachycardia induced by $BaCl_2$. Additionally, although the incidence of mortality was significantly reduced following administration of terfenadine and amiodarone, a high dose of terfenadine (18 mg/kg) was more lethal than a moderate dose of terfenadine (12 mg/kg) in the $BaCl_2$-induced as well as in the aconitine-induced VT. Furthermore, terfenadine and amiodarone as K^+ channel agonists have been widely investigated, whereas little information is available on the antiarrhythmic effects of terfenadine and amiodarone as Na^+ and Ca^{2+} channel blockers. More complex mechanisms may be involved in the protective role of terfenadine on the arrhythmia model, and elucidation of the effects requires further studies.

In humans, terfenadine is well absorbed and metabolized in liver microsomes to form hydroxyterfenadine. Hydroxyterfenadine then undergoes subsequent oxidation to the corresponding carboxylic acid, which is considered to be the biologically active antihistamine (35). This carboxylic acid has been proved no effect on K^+ channels in ventricular myocytes, even at high plasma levels (36). However, terfenadine itself produced cardiotoxicity in isolated rabbit hearts and human atrial myocytes (4). CYP3A4 has been demonstrated to be the principal cytochrome P450 enzyme involved in the metabolism of terfenadine, and the adverse clinical drug interactions of terfenadine have been associated with the inhibition of its CYP3A4-mediated metabolism (36). Drugs including ketoconazole and erythromycin may inhibit the activity of CYP3A4, which leads to an increase in terfenadine plasma levels and finally produces toxicity in the heart. The present data indicated that terfenadine dose-dependently prolonged the QTc interval, and this effect was similar to that of amiodarone. Furthermore, similar side effects occurred following overdose of antiarrhythmic drugs, including quinidine and sotalol. Therefore, the QTc interval prolongation caused by terfenadine may be similar to the adverse effects of several antiarrhythmic drugs.

Amiodarone is one of the few remaining treatment options for ventricular arrhythmia and for reducing the incidence of atrial fibrillation, particularly in heart failure patients with severely impaired left ventricular function, where class I antiarrhythmic drugs or dronedarone are considered as contra-indicated (37-39). The present study demonstrated that terfenadine and amiodarone may not only similarly delay the onset time of ventricular arrhythmia induced by $BaCl_2$, but also increase the cumulative dosage of aconitine required to induce VPC, VT, VF and CA. Furthermore, the two drugs were equally efficient in shortening the duration of ventricular arrhythmia. Aside from cardiotoxicity, the accumulation of amiodarone may also lead to thyroid dysfunction and pulmonary fibrosis (40,41). Since these adverse drug reactions have limited the application of amiodarone, the development of new antiarrhythmic drugs is urgently required. The present study provided preliminary evidence that terfenadine had potential antiarrhythmic effects, which greatly facilitates the discovery and development of novel antiarrhythmic drugs.

Several limitations of the present study should be noted. The investigation was performed only in ventricular arrhythmia animal models, the antiarrhythmic activity of terfenadine should be confirmed in cardiomyocytes through *in vitro* experiments. The preventative and therapeutic effects of terfenadine were only detected in ventricular antiarrhythmia through comparing with the K^+ channel blocker amiodarone. Since terfenadine may potentially inhibit Na^+ channels and L-type Ca^{2+} channels, similar comparisons with Na^+ channels and Ca^{2+} channel blockers should be conducted to determine the antiarrhythmic effect of terfenadine. The present investigation was performed following anesthetizing of the animals, which may have had an effect on ECG parameters. Whether terfenadine may be suitable as a novel antiarrhythmic drug requires additional studies.

In conclusion, it was identified that terfenadine and amiodarone similarly prolonged the QTc interval in rats. In $BaCl_2$/aconitine induced ventricular arrhythmia models, terfenadine and amiodarone not only similarly delayed the onset time of arrhythmia induced by barium chloride (all $P<0.05$), but also increased the cumulative dosage of aconitine required to induce various types of arrhythmia. Furthermore, the two drugs

equivalently caused a significant decrease in the duration of VT. The potential antiarrhythmic effect of terfenadine was shown to not be inferior to that of amiodarone in experimental ventricular arrhythmia rat models.

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