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The Acute Effects Of ATP-Sensitive Potassium Channel Opener (Pinacidil) And Blocker (Glimepride) On the Ischemia or Reperfusion-Induced Arrhythmias

ATP-Bağımlı Potasyum Kanal Açıcısı Pinasidil ve Blokeri Glimeprid'in İskemi ve Reperfüzyon ile Uyarılan Aritmiler Üzerine Akut Etkisi

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Abstract

Objective: Myocardial ischemia generated by coronary occlusion and myocardial reperfusion by the opening of occluded coronary vessel in the acute stage leads to lethal arrhythmia and sudden death in humans. That is why pharmacological drug research to decrease these arrhythmias have been researched intensively. In this study, the effect of acute administration of pinacidil and glimepride, on ischemia or reperfusion-induced arrhythmia was aimed to be researched.

Materials and Methods: Two groups were designed; In the first group, only ischemia was produced by the ligation of the left coronary artery in 20 minutes, and in the second group 6 minutes of ischemia by the ligation of the artery and the subsequent 15 minutes of reperfusion were produced by the opening of the occluded artery. Drugs were administered intravenously at 2 minutes of ischemia in the first group and just following the reperfusion in the second group. The ECG and blood pressure were recorded during ischemia and reperfusion period. The type, duration, incidence of arrhythmia, heart rate, blood pressure, and the death rate from the recording were calculated. All data were first compared by one-way ANOVA. Then, the drug groups with their control, and control and drug groups with each other were compared by a one-tailed t-test. The incidence of arrhythmia and the death rate between groups was compared by the Ki square test.

Results: Pinacidil significantly decreased the arrhythmia score both in the ischemia and reperfusion period but glimepiride was not effective when they were given intravenously in the acute stage of ischemia or reperfusion.

Conclusion: This study suggests that pinacidil might be a candidate for drugs that can be used to decrease arrhythmia in the acute stage of myocardial infarction but more study is needed to reveal the antiarrhythmic or proarrhythmic effect of glimepride with different doses in the acute stage of myocardial infarction.

Keywords: Ischemia, Reperfusion, Arrhythmia, Pinacidil, Glimepiride.

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Öz

Amaç: Miyokart enfarktüsünün akut döneminde koroner damar tıkanmasına bağlı olarak oluşan miyokardiyal iskemi ve arkasından damarın açılmasıyla oluşan reperfüzyon insanlarda öldürücü aritmilerin oluşmasına ve ani ölümlere yol açmaktadır. Bu nedenle bu aritmilerin azaltılması için farmokolojik ilaç araştırmaları yoğun bir şekilde yapılmaktadır. Bu çalışmada pinasidil ve glimepridin akut uygulamasının iskemi ve-reperfüzyon aritmileri üzerine etkisinin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: İki grup oluşturulmuştur; Birinci grupta sol koroner arter 20 dakika süreyle bağlanarak sadece iskemi oluşturulmuş, ikinci grupta damar bağlanarak 6 dakika iskemi ve arkasından tıkanan damar açılarak 15 dakika reperfüzyon yapılmıştır. İlaçlar intravenöz olarak birinci grupta, iskeminin ikinci dakikasında, ikinci grupta, hemen reperfüzyondan sonra uygulanmıştır. İskemi ve reperfüzyon süresince EKG ve kan basıncı kaydedilmiştir. Kayıtlardan aritmilerin tipi, süresi ve yoğunlukları, kalp atım hızı, kan basıncı ve ölüm oranı belirlenmiştir. Bütün veriler tek yönlü ANOVA ile karşılaştırılmıştır. Daha sonra ilaç grupları kendi kontrol gruplarıyla, ilaç ve kontrol grupları kendi aralarında ikili olarak tek kuyruklu t testi ile karşılaştırılmıştır. Aritmi yoğunlukları ve ölüm oranları Ki kare testi ile karşılaştırılmıştır.

Bulgular: İskemi ve reperfüzyon sırasında intravenöz olarak verildiklerinde, pinasidil hem iskemi, hemde reperfüzyon periyodunda belirgin bir şekilde aritmi skorunu azaltmış, glimeprid ise etkisiz kalmıştır.

Sonuç: Bu çalışma pinasidilin miyokart enfarktüsünün akut döneminde oluşan aritmileri azaltmada etkili bir ilaç olabileceğini ortaya koymuştur. Ancak glimepridin miyokart enfarktüsünün akut döneminde antiaritmik ya da proaritmik etkisinin ortaya konması için farklı dozları ile daha fazla çalışma gerekmektedir.

Anahtar Kelimeler: İskemi, Reperfüzyon, Aritmi, Pinasidil, Glimeprid

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Introduction

Acute myocardial infarction (AMI) is still a common cause of sudden death in humans. Severe arrhythmia appearing in the first minutes or hours of AMI is the major cause of death (1). The treatment in the acute phase of AMI has been usually mediated by thrombolytic drugs or by antiarrhythmic drugs. In the experimental studies, the ATP-dependent channel blockage or opening was observed to be effective in decreasing the arrhythmias and death of animals following ischemia and reperfusion (2). Glimepiride is a potassium channel blocker and has been used in diabetic patients to increase insulin release (3). It was indicated that the death due to cardiovascular disease is lesser in patients who used glimepride (4). In animal studies, ıt was shown that glimepride blocks the sarcolemmal ATP dependent potassium channel but not the mitochondrial ATP dependent potassium channel (5) and induces NO release (6) and has fewer effects to decrease myocardial blood flow and contraction than other sulfonylurea drugs, glibenclamide (7). In the previous animal study, glimepride administered before coronary ligation was shown to decrease the ventricular arrhythmia observed following myocardial ischemia and reperfusion (8, 9). Pinacidil has been used as a hypotensive drug in clinics (10,11). Pinasidil is ATP dependent potassium channel opener and induces early repolarisation of a myocardial cell (12) and decreases contraction (13). In animal studies, the pretreatment of pinacidil was found to decrease ventricular arrhythmia that occurred during myocardial ischemia and reperfusion (2,14,15). But there are also some studies indicating the proarrhythmic effect of pinacidil (16). Clinically the treatment of arrhythmia by drugs has been performed during the early ischemia or reperfusion period of AMI. One previous study suggested that pinacidil by shortening the action potential in normal myocardial cells and glimepride by increasing action potential duration in ischemic cells may decrease heterogeneity in action potentials and produce an antiarrhythmic effect (17). This study aims to observe the effect of acute administration of pinacidil and glimepride on ischemia or reperfusion-induced arrhythmias and the survival of the animal.

Materials and Methods

Animals

In this study, 80 male Sprague Dawley rats 6-7 months old were used. Animals were grown 12L/12D photoperiods and fed with food and water ad libitum. All animals were experimented with adherence to all guiding principles in the care and use of animals with the recommendation from the declaration of Helsinki and approved by the local animal ethic committee of Abant Izzet Baysal University (Protocol number; 2019-21/53, 2019-24/51). The ethical approval for the study were obtained for ischemia (2019/24), and ischemia-reperfusion groups (2019/21) individually. Two groups of the animal were designed as ischemia and ischemia-reperfusion groups. The drug-control and drug-treated groups were defined as a subgroup in ischemia and ischemia-reperfusion group.

Surgical Operation

All surgical operations were performed under the urethane (Sigma Aldrich, Interlab/Türkiye) (2gr/kg) anesthesia. Tracheostomy for artificial respiration, the right carotid artery cannulation for blood pressure measurement, thoracotomy between 4.-5. intercostal space for coronary artery ligation was performed. In the first group, the coronary artery was ligated 2 mm away from its origin, aorta to produce 20 minutes of ischemia. Drugs were given from the tail vein at 2 minutes following coronary ligation in this group. In the second group, six minutes of ischemia by the ligation of coronaries, following 15 minutes of reperfusion by the releasing of ligature were produced. Drugs were given just following the reperfusion in this group. At the end of ischemia or reperfusion, the respirator was cut off, and animals having spontaneous reflex respiration were accepted as live animals. The heart of these animals was ablated from the thorax and perfused from the aorta for the determination of the risk of the infarct zone. The arrhythmia and other values including blood pressure, heart rate, the type and duration of arrhythmia recorded during ischemia,

and reperfusion were evaluated. Animals having no spontaneous respiration at the end of ischemia were accepted as dead animals and the arrhythmia data obtained in these animals were not evaluated.

Drugs

The dose of pinacidil and glimepride used in this study were chosen according to the previous research work (3, 12); Pinacidil (Sigma Aldrich, Interlab/Türkiye) in 0.1mg/ml/kg, glimepiride (Sigma Aldrich, Interlab/Türkiye) in 1mg/ml/kg. In control animals, the only solvent of the drug (saline for pinacidil and DMSO ¼ + Etil alcohol ¼ + saline solution 2/4 for glimepride)) was administered. Drugs were prepared daily and given intravenously from the tail vein.

Recording And Evaluation of Arrhythmia

Lead II of ECG and blood pressure were recorded during ischemia and reperfusion by a computerized recording system (Biopac System Inc, USA; Türkiye representative: Commat Ltd, Ankara) using Pro 3.7 software. The duration, type, and the incidence of arrhythmia were determined from recorded ECG and the arrhythmias were evaluated according to the Lambeth convention (18). Blood pressure and heart rate were determined at 1,3,5,10 and 15 minutes following coronary artery ligation in the first group and 1,3,5,10,15 minutes following ischemia or reperfusion in the second group.

Determination of Risk of Infarct Zone

Before the heart was excised out together with the aorta, diluted heparin solution (1/10) was given intravenously from the cannulated carotid artery. To demarcate the ischemic and nonischemic zone of the heart, the aorta was perfused first with physiologic saline solution and then ethyl alcohol. Following perfusion with the alcohol, the color of perfused myocardium changes to white, and the ischemic portion remain pinkish. All procedures used for the determination of the risk of infarct zone were done according to the Lepran et al (19).

Biochemical Analysis

Blood glucose levels before coronary ligation and at the end of the experiment were determined by a glucometer from a blood sample taken from the tail vein. Blood sugar at the end of the experiment in the dead animals at the end of 15 minutes of reperfusion in I/R groups and the end of 20 minutes of ischemia in the ischemia group were not measured.

Statistical Analysis

The mean and standard error for the duration of arrhythmia, total arrhythmia, heart rate, blood pressure, the weight of the heart, and the risk of infarct zone were determined and compared by one-way ANOVA. Then the drug and drug control group were compared by one tail t-test. The incidence of all type of arrhythmia between drug and drug control groups were compared by the Ki square test.

Results

ST-segment elevations and the changes in the amplitude of QRS were observed following coronary ligation. The values including the weight of animals, the weight of the infarct zone and the percentage of risk of infarct zone, and the blood glucose level in each group were presented in table 1 (Ischemia group) and table 2 (Reperfusion group). There was no significant difference between drug control and drug-treated groups in the blood pressure during 20 minutes of ischemia in the first group, figure 1, and 6 minutes of ischemia / 15 minutes reperfusion in the second group, figure 2. There were also no significant differences in the heart rate between the drug control and drug-treated group during 20 minutes of ischemia, table 3 and 15 minutes of reperfusion, table 4. There was no significant difference in arrhythmia score between the glimepride treated group and its control in the ischemia group, table 5. But in ischemia reperfusion group, nonsignificant increament in the score of arrhythmias was observed in glimepride treated group in respect

to its control, table 6. Pinacidil significantly decreased the arrhythmia scores in the ischemia group, table 5, and in the I/R group in respect to its controls, table 6. Similarly, pinacidil significantly, glimepride nonsignificantly decreased total arrhythmia observed in the ischemia group, table 5. Total arrhythmia and ventricular premature contraction during reperfusion in the I/R group were significantly higher in glimepride treated rats, and VT and VF were lower in pinacidil treated rats than pinacidil control, table 6. In the I/R group, the incidence of ventricular premature contraction and ventricular tachycardia was also higher in glimepride treated rats, but lower in pinacidil treated rats regarding their control, table 7. The incidence of ventricular tachycardia in the ischemia group was higher in glimepride treated animals but lower in the pinacidil treated group in respect to its control, table 8. The death of animals at the end of 20 minutes of ischemia was more in glimepiride treated animals but lesser in pinacidil treated animals regarding to their control. The death rate did not change significantly in the I/R group after both pinacidil and glimepride treatment.

Tablo 1

Body weight, risk of enfarct zone and blood glucose level in animals used in ischemia group (Mean ± Standart error).

Groups	Body weight (gr)	Risk of infarct zone (gr)	Risk of infarct zone (%)	Blood glucose before ligation, mg/dl	Blood glucose at the end of ischemia period mg/dl
Glimepride Control (a)	361 ± 44 (n=5)	0,50 ± 0,03 (n=5)	43 (n=5)	94 ± 15 (n=5)	65 ± 8 (n=5)
Glimepride Drug (b)	394 ± 14 (n=6)	0,51 ± 0,06 (n=6)	45 (n=6)	106 ± 19 (n=6)	91 ± 25 (n=3)
Pinacidil Control (c)	345 ± 25 (n=6)	0,41 ± 0,03 (n=6)	40 (n=6)	131 ± 31 (n=5)	139 (n=1)
Pinacidil Drug(d)	342 ± 12 (n=8)	0,46 ± 0,02 (n=8)	47 (n=8)	112 ± 44 (n=8)	112 ± 30 (n=6)

n: Number of animals

Table 2

Body Weight, Risk of Enfarct Zone and Blood Glucose Level in Animals Used in Ischemia-Reperfusion Group (Mean ± Standart error).

Groups	Body weight (gr)	Risk of infarct zone (gr)	Risk of infarct zone (%)	Blood glucose before ligation mg/dl	Blood glucose at the end of reperfusion period mg/dl
Glimepiride Drug Control (a)	319 ± 15 (n=7)	0,44 ± 0,02 (n=7)	42 (n=7)	112± 21 (n=6)	68 ± 18 (n=5)
Glimepiride Drug (b)	398 ± 26 (n=7)	0,52 ± 0,02 (n=7)	45 (n=7)	125 ± 33 (n=7)	63 ± 15 (n=5)
Pinacidil Drug Control (c)	313 ± 19 (n=7)	0,37 ± 0,06 (n=7)	39 (n=7)	122 ± 21 (n=7)	99 ± 26 (n=7)
Pinacidil Drug (d)	342 ± 27 (n=6)	0,51 ± 0,01 (n=6)	51 (n=6)	104 ± 11 (n=6)	78 ± 7 (n=6)

n: Number of animals

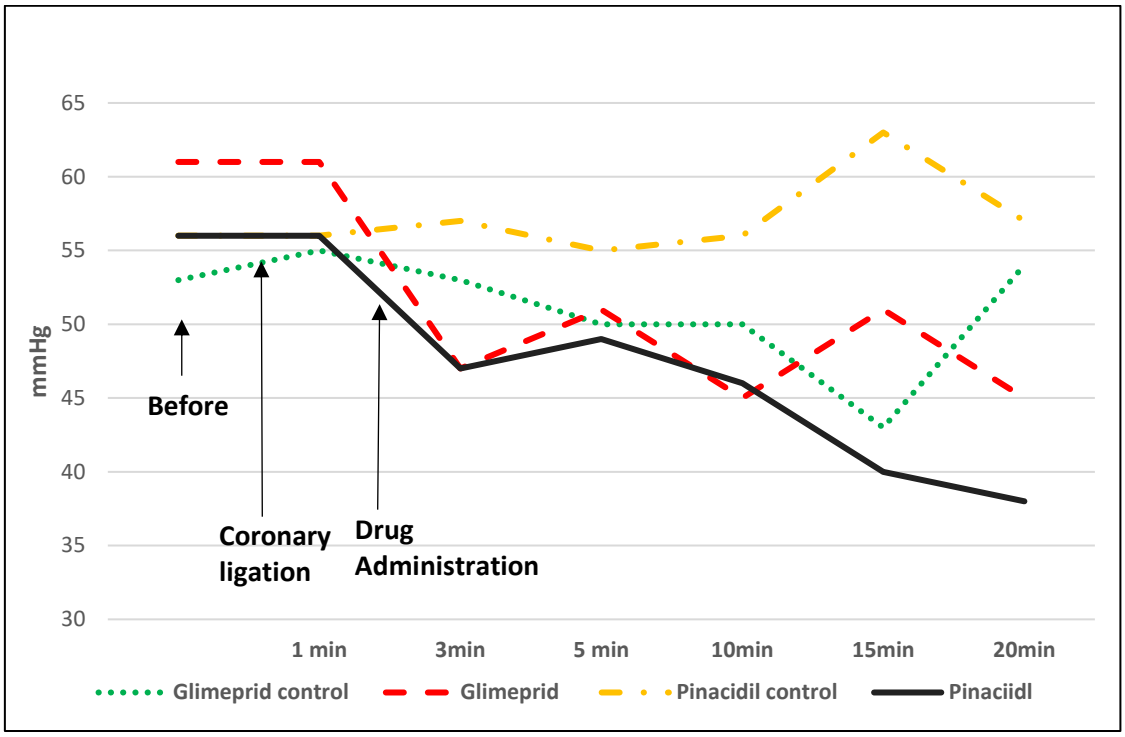

Figure 1. The blood pressure before and following coronary ligation during 20 minutes in ischemia group.

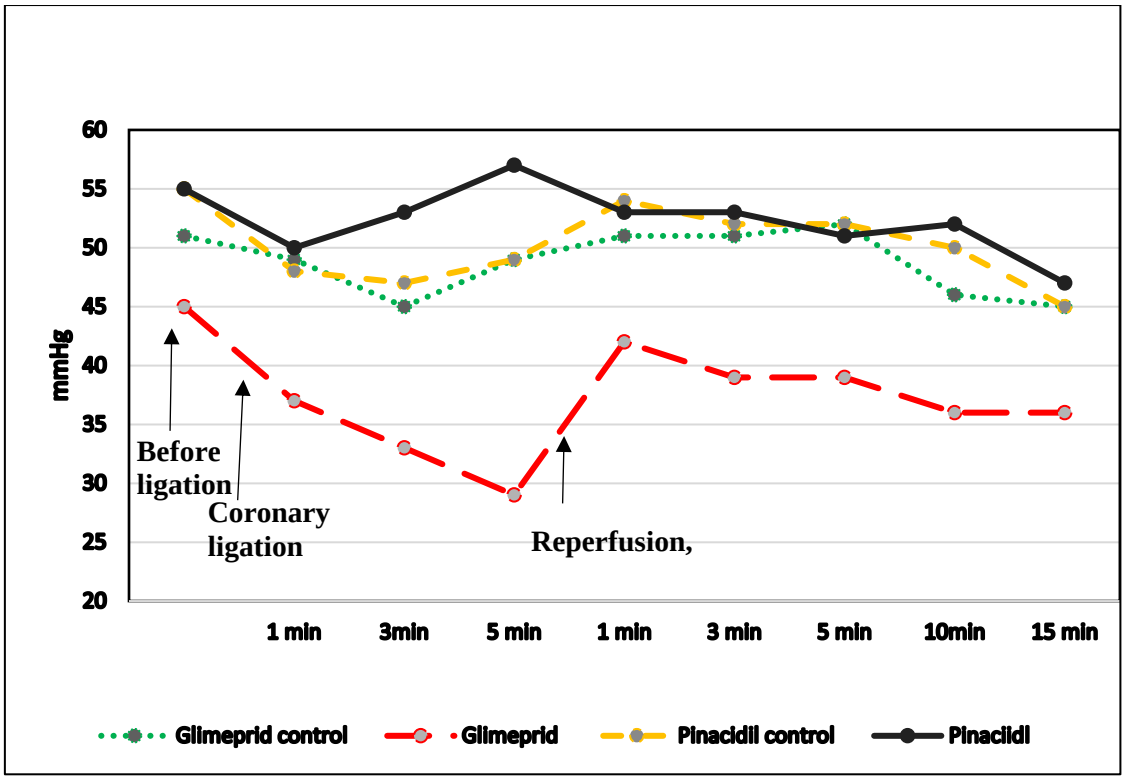

Figure 2. The blood pressure before and following 6 minutes ischemia and 15 minutes reperfusion in ischemia-reperfusion group.

Tablo 3

Heart Rate Before and During Ischemia in Ischemia Group, Beat/Minute (Mean ± Standart Error).

	Before coronary ligation	Following coronary ligation					
		1 min	3 min	5 min	10 min	15 min	20 min
Glimepride Drug Control (a)	300 ± 41 (n=6)	273 ± 43 (n=5)	262 ± 38 (n=5)	259 ± 41 (n=5)	263 ± 49 (n=4)	275 ± 40 (n=5)	314 ± 16 (n=3)
Glimepride Drug (b)	290 ± 24 (n=6)	285 ± 20 (n=5)	268 ± 33 (n=4)	294 ± 18 (n=5)	240 ± 62 (n=3)	261 ± 17 (n=2)	279 ± 15 (n=3)
Pinacidil Drug Control (c)	381 ± 18 (n=6)	345 ± 34 (n=3)	387 ± 29 (n=4)	384 ± 29 (n=4)	387 (n=1)	368 ± 44 (n=2)	364 ± 49 (n=2)
Pinacidil Drug (d)	334 ± 24 (n=8)	324 ± 19 (n=7)	305 ± 25 (n=7)	323 ± 17 (n=6)	322 ± 17 (n=6)	299 ± 19 (n=6)	301 ± 20 (n=6)

n: Number of animals

Tablo 4

Heart Rate Before and Following Ischemia-Reperfusion in Ischemia-Reperfusion Group, Beat/Minute (Mean ± Standart Error), *P<0,05, **P<0,01, **A: Diffference from Glimeprid Control

	Before Ligation	Following coronary ligation			Following reperfusion			
		1 min	3 min	5 min	1 min	5 min	10 min	15 min
Glimepride Drug Control (a)	315 ± 12 (n=7)	320 ± 7 (n=6)	265 ± 36 (n=6)	298 ± 28 (n=6)	275 ± 36 (n=6)	283 ± 36 (n=6)	281 ± 40 (n=6)	282 ± 50 (n=5)
Glimepride Drug (b)	304 ± 23 **a (n=7)	298 ± 30 **a (n=5)	300 ± 28 (n=5)	282 ± 44 (n=5)	277 ± 25 (n=5)	322 ± 22 (n=5)	319 ± 25 (n=6)	293 ± 24 (n=5)
Pinacidil Drug Control (c)	333 ± 17 (n=7)	346 ± 18 (n=7)	318 ± 28 (n=7)	335 ± 21 (n=7)	335 ± 21 (n=7)	329 ± 19 (n=7)	329 ± 21 (n=7)	310 ± 22 (n=7)
Pinacidil Drug(d)	354 ± 13 (n=6)	338 ± 9 (n=6)	302 ± 30 (n=6)	330 ± 11 (n=6)	318 ± 10*c (n=6)	310 ± 14 (n=6)	316 ± 17 (n=6)	306 ± 18 (n=6)

n: Number of animals

Table 5

The Type, Duration, And Score of Arrhythmia During 20 Minutes of Ischemia, (Mean ± Standart Error) *P<0,05, **P<0,01, *C: Difference from Pinacidil Control

	VPC (Sec)	VT (Sec)	VF (Sec)	Total arrhythmia (Sec)	Bradycardia (Sec)	Score of arrhythmia
Glimepride Drug Control (a)	88,2 ± 51,1 (n=5)	3,6 ± 3,6 (n=5)	24,2 ± 20,8 (n=5)	116 ± 45,3 (n=5)	314,6 ± 193 (n=5)	4,0 ± 0,6 (n=5)
Glimepride Drug (b)	60,5 ± 14,0 (n=4)	8,0 ± 6,4 (n=4)	1,7 ± 1,7 (n=4)	70,5 ± 12,6 (n=4)	269,7 ± 269,7 (n=4)	4,1 ± 0,6 (n=6)
Pinacidil Drug Control (c)	96,5 ± 4,5 (n=2)	5,3 ± 2,7 (n=2)	0,0 (n=2)	101,3 ± 1,2 (n=2)	0,0 (n=2)	5,3 ± 1,0 (n=6)
Pinacidil Drug (d)	27,6 ± 11,8 (n=6)	2,6 ± 1,8 (n=6)	11,9 ± 11,9 (n=6)	42,3 ± 21,4*c (n=6)	11,9 ± 11,9 (n=6)	3,1 ± 0,8*c (n=8)

VPC; Ventricular premature contraction, VT; Ventricular tachycardia, VF; Ventricular fibrillation
n: Number of animals

Table 6

The Type, Duration, And Score of Arrhythmia During 15 Minutes of Reperfusion Following 6 Minutes of Ischemia, (Mean ± Standart Error) *P<0,05, **P<0,01, *A: Difference from Glimeprid Control, *C: Difference from Pinacidil Control

	VPC (Sec)	VT (Sec)	VF (Sec)	Total arrhythmia (Sec)	Bradycardia (Sec)	Score of arrhythmia
Glimepride Drug Control (a)	4,1 ± 2,7 (n=6)	1,1 ± 1,1 (n=6)	1,8 ± 1,9 (n=6)	7,1 ± 5,5 (n=6)	155,8 ± 155,8 (n=6)	1,7 ± 0,99 (n=7)
Glimepride Drug (b)	62,6 ± 59,1 *a (n=5)	1,2 ± 0,8 (n=5)	0,0 ± 0,0 (n=5)	63,8 ± 59,3 *a (n=5)	4,2 ± 4,2 (n=5)	2,8 ± 1 (n=7)
Pinacidil Drug Control (c)	4,0 ± 2,8 (n=7)	2,2 ± 1,8 (n=7)	0,4 ± 0,4 (n=7)	6,7 ± 4,7 (n=7)	0,0 ± 0,0 (n=7)	1,1 ± 0,6 (n=7)
Pinacidil Drug (d)	1,5 ± 1,5 (n=6)	0,0 ± 0,0 *c (n=6)	0,0 ± 0,0 *c (n=6)	1,5 ± 1,5 (n=6)	0,0 ± 0,0 (n=6)	0,1 ± 0,1 *c (n=6)

VPC; Ventricular premature contraction, VT; Ventricular tachycardia, VF; Ventricular fibrillation
n: Number of animals

Table 8

The Death Rate and The Incidence of Arrhythmia at The End Of 20 Minutes of Ischemia in Rats. Mean ± Standart Error, *P<0,05, **P<0,01, **A: Difference from Glimeprid Control, *C: Difference from Pinacidil Control

Groups	N	Death rate (N) / %	Arrhythmia, N/%			BradycardiaN/ %
			VPC	VT	VF	
Glimepride Drug Control (a)	5	0 / 0	4 / 67	1 / 17	2 / 33	3 / 50
Glimepride Drug (b)	6	2 / 33**a	6 /100	4/ 67**a	2 / 33	3 / 50
Pinacidil Drug Control (c)	6	4 / 67	6 / 100	4 / 67	3 / 86	2 / 33
Pinacidil Drug (d)	8	2 / 25*c	6 / 75	2 / 25*c	1 / 13*c	3 / 38

VPC; Ventricular premature contraction, VT; Ventricular tachycardia, VF; Ventricular fibrillation, N; Number of animals

Discussion

In several previous studies, the antiarrhythmic or proarrhythmic effect of ATP-dependent potassium channels in experimentally produced myocardial ischemia or reperfusion was observed in animals (2,13,20). ATP-dependent channel opener, pinacidil (2,13, 21,22), and glimepride (8) were shown to be effective to decrease ischemia or reperfusion-induced arrhythmia. In these studies, drugs were given to animals intravenously or intraperitoneally before coronary ligation. Similarly to some of previous study indicated above, pinacidil was found to be effective to decrease ventricular type of arrhythmia when it was administered in the acute stage of ischemia and also at reperfusion period. However, glimepride was not found to be effective in decreasing the arrhythmia observed during 20 minutes of ischemia, but oppositly glimepride administered at the start of reperfusion nonsignificantly increased the arrhythmia. The antiarrhythmic effect of pinacidil observed in this study may be due to its vasodilator effect or decreasing heterogeneity in action potential duration between ischemic and nonischemic cells. Pinacidil induces NO release and activates the Na/H+ exchanger (23). Vasodilator effect of pinacidil may increase blood flow via the collateral to the ischemic myocardium and decrease ischemia-induced damage and arrhythmia. Pinacidil was observed to increase coronary blood flow during ischemia and reperfusion even in very low doses (24). Antiarrhythmic effect of pinacidil in the acute stage may also be caused by decreased calcium overload in myocardial cell (25). Calcium overload increases in myocardial cells in prolonged action potential and increase contraction and oxygen usage. This might be the cause of increased reperfusion induced arrhythmia in glimepride treated group. Otherwise pinacidil decreases calcium overload and contraction and myocardial oxygen usage and increases resistance against ischemic injury. Early after depolarization (EAD) is one of the causes of arrhythmia occurred following ischemia or reperfusion. Pinacidil was shown to decrease EAD with abnormal automaticity generated in the Purkinje cell (26). Pinacidil decreases the action potential and duration of QT interval (27, 28) and increases the recovery of action potentials in ischemic cells (29). The heterogeneity in action potentials occurring in ischemic and

non-ischemic cells is a major cause of arrhythmia. Antiarrhythmic effect of ATP dependent potassium channel opener even blocker may depend on their effect to decrease heterogeneity in action potentials. Heterogeneity in action potentials duration was observed even between epicardial and endocardial nonischemic myocardial cells and pinacidil decreased this heterogeneity (30). It was also observed that pinacidil decreased the action potential duration in epicardial cells more than the endocardial cells (31). The elevation of the ST segment that occurs just the following ischemia was not observed after pinacidil administration (32). All of these effects of pinacidil mentioned above might be responsible for less arrhythmia observed during ischemia or reperfusion in the present study.

Glimepiride is an antidiabetic drug used in the treatment of diabetes. It was shown that cardiovascular disease and morbidity are lesser in patients using glimepride than the other sulphonylurea drugs (33). It was also indicated that glimepride increases coronary sudden death and decreases the incidence of ventricular arrhythmia (34). In the present study, glimepride was not effective to decrease or increase arrhythmia during 20 minutes of ischemia, but similarly, it increased the death of animals. There are a few studies investigating the pretreatment effect of glimepride on ischemia-reperfusion-induced arrhythmia (9) and there was no study found on the acute effect of glimepride on ischemia or reperfusion-induced arrhythmia. Glimepride is ineffective to block the preconditioning effect of ischemia even potentiating the preconditioning effect of ischemia concerning other sulphonylurea drugs such as glibenclamide (35). Glimepride blocks the sarcolemmal ATP dependent potassium channel but without effect on the mitochondrial ATP dependent potassium channel and this effect of glimepiride was shown to block by pinacidil (5). The blockage of the sarcolemmal ATP-dependent potassium channel is more effective to decrease ventricular arrhythmia than the blockage of the mitochondrial ATP-dependent potassium channel (36). It was found that glimepride increases NO production from an embryonic stem cell (6). It was also shown that glimepride is less effective than glibenclamide to decrease coronary blood flow and contraction (37). All of these mentioned above studies support there is a protective effect of glimepride against ischemia. In our study, a nonsignificant protective effect of glimepride against arrhythmia was seen during 20 minutes ischemic period, but it did not have a significant effect on the score of arrhythmia. There was also no significant effect of acute administration of glimepride on the arrhythmia when it was given at reperfusion period. The reason for the ineffectiveness of glimepride on reperfusion-induced arrhythmia might be due to the time and route of administration. In a previous study, glimepiride was given intraperitoneally before coronary ligation, but in this study, it was administered intravenously (9). The time, route, and dose of glimepride or pinacidil may be a factor in decreasing or increasing arrhythmia. The action potentials in ischemic cells shorten and pinacidil may decrease heterogeneity in AP duration between the ischemic and non-ischemic regions and decrease arrhythmia. But glimepiride given during ischemia will not be effective on the normoxic myocardial cells, because ATP-dependent potassium channels in normal myocardial cells are in a closed state. Glimepiride administered following coronary ligation was possibly ineffective on the ischemic cells, because glimepride given just the following ligation could not be reached the ischemic region. In reperfusion, the heterogeneity in AP of myocardial cells in the reperfused region is higher and that's why normally more severe ventricular arrhythmia is seen in this period (38). Pinacidil increases blood flow to the normal and reperfused myocardial region and recovers the ischemic myocardial cell earlier. Since pinacidil decreases heterogeneity in AP duration, the ventricular arrhythmia and the score of arrhythmia decreased during the reperfusion period. Glimepride was expected to decrease the ventricular arrhythmia and the score of arrhythmia in the reperfusion period, but it did not. A possible explanation for that glimepiride increases heterogeneity in AP duration, especially during reperfusion, and induces more ventricular tachycardia and ventricular premature contraction. Glimepride used in a 1mg/kg dose may be higher and may increases heterogeneity in AP duration of myocardial cells more in the reperfused region and that may be the cause of more ventricular arrhythmia observed in the I/R group.

Conclusion

Pinacidil decreases arrhythmia when it was given in the acute stage of ischemia or reperfusion. Glimepiride was nonsignificantly effective in decreasing ventricular arrhythmias when it was administered in the ischemic period but not effective on the arrhythmia when it was administered reperfusion period. More studies are needed specially to find the effective dose of glimepride on ischemia or reperfusion-induced arrhythmia.

Study Limitation: The number of animals used in this study was limited to 10 animals for each group. Dose-dependent effect of pinacidil and glimepiride on the ischemia-reperfusion arrhythmias could not be studied.

Ethics Committee Approval: The study was approved by the local animal ethic committee of Abant Izzet Baysal University (Protocol numbers: 2019-21/53, 2019-24/51).

Informed Consent: N/A.

Conflict of Interest: Authors declared no conflict of interest.

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References

1. Sara JD, Eleid MF, Gulati R, Holmes DR Jr. Sudden cardiac death from the perspective of coronary artery disease. Mayo Clinic Proceedings 2014; 89(12):1685-98.
2. Gonca E, Bozdogan O. Both mitochondrial KATP channel opening and sarcolemmal KATP channel blockage confer protection against ischemia/reperfusion-induced arrhythmia in anesthetized male rats. Journal of Cardiovascular Pharmacology and Therapeutics 2010;15(4): 403-411.
3. Basit A, Riaz M, Fawwad A. Glimepiride: evidence-based facts, trends, and observations (GIFTS). Vascular Health and Risk Management 2012; 8: 463-472.
4. Aravind, S. R., Mittal, S., Venkatraman, S., Deka, N., Parmar, G., Amarnath, S., & Mohan, V. Cardiovascular Profile of Modern Sulfonylureas: Focus on Glimepiride. Journal of the Association of Physicians of India 2019; 67: 17
5. Mocanu MM, Maddock HL, Baxter GF, Lawrence CL, Standen NB, Yellon DM. Glimepiride, a novel sulfonylurea, does not abolish myocardial protection afforded by either ischemic preconditioning or diazoxide. Circulation 2001; 103(25): 3111-3116.
6. Ueba H, Kuroki M, Hashimoto S, Umemoto T, Yasu T, Ishikawa SE, Saito M, Kawakami M. Glimepiride induces nitric oxide production in human coronary artery endothelial cells via a PI3-kinase-Akt dependent pathway. Atherosclerosis 2005; 183(1): 35-39.
7. Végh A, Papp JG. Hemodynamic and other effects of sulphonylurea drugs on the heart. Diabetes Research and Clinical Practice 1996; 31: (Suppl 2); S43-53.
8. Bozdogan, O., Leprán, I., & Papp, J. G. Effect of glimepiride and glibenclamide, inhibitors of ATP-dependent K (+)-channel, on ischemia-reperfusion induced arrhythmias in rats. Acta Physiologica Hungarica 1996; 84(3): 265-266
9. El-Reyani NE, Bozdogan O, Baczkó I, Leprán I, Papp JG. Comparison of the efficacy of glibenclamide and glimepiride in reperfusion-induced arrhythmias in rats. European Journal of Pharmacology 1999; 365(2-3): 187-192.

10. Goldberg MR. Clinical pharmacology of pinacidil, a prototype for drugs that affect potassium channels. Journal Cardiovascular Pharmacology 1988; 12 (Suppl 2): S41-47.
11. Fish FA, Prakash C, Roden DM. Suppression of repolarization-related arrhythmias in vitro and in vivo by low-dose potassium channel activators. Circulation 1990; 82(4): 1362-1369.
12. Fedorov VV, Glukhov AV, Ambrosi CM, Kostecki G, Chang R, Janks D, Schuessler RB, Moazami N, Nichols CG, Efimov IR. Effects of KATP channel openers diazoxide and pinacidil in coronary-perfused atria and ventricles from failing and non-failing human hearts. Journal of Molecular and Cellular Cardiology 2011; 51(2): 215-225.
13. Cole WC, McPherson CD, Sontag D. ATP-regulated K+ channels protect the myocardium against ischemia/reperfusion damage. Circulation Research 1991; 69(3): 571-581.
14. Leprán I, Baczkó I, Varró A, Papp JG. ATP-sensitive potassium channel modulators: both pinacidil and glibenclamide produce antiarrhythmic activity during acute myocardial infarction in conscious rats. Journal of Pharmacology and Experimental Therapeutics 1996 Jun; 277(3):1215-20.
15. Antzelevitch, C., & Di Diego, J. M. Role of K+ channel activators in cardiac electrophysiology and arrhythmias. Circulation 1992; 85(4): 1627-1629
16. D'Alonzo AJ, Zhu JL, Darbenzio RB, Dorso CR, Grover GJ. Proarrhythmic effects of pinacidil are partially mediated through enhancement of catecholamine release in isolated perfused guinea-pig hearts. Journal of Molecular and Cellular Cardiology 1998; 30(2): 415-23.
17. Yaşar S, Bozdoğan Ö, Kaya ST, Orallar HS. The effects of ATP-dependent potassium channel opener; pinacidil, and blocker; glibenclamide, on the ischemia-induced arrhythmia in partial and complete ligation of coronary artery in rats. Iranian Journal of Basic Medical Sciences 2015;18(2):188-193.
18. Curtis MJ, Hancox JC, Farkas A, Wainwright CL, Stables CL, Saint DA, Clements-Jewery H, Lambiase PD, Billman GE, Janse MJ, Pugsley MK, Ng GA, Roden DM, Camm AJ, Walker MJ. The Lambeth Conventions (II): guidelines for the study of animal and human ventricular and supraventricular arrhythmias. Pharmacol Ther. 2013 Aug;139(2):213-48.
19. Leprán, I., Koltai, M., & Szekeres, L. Coronary artery ligation, early arrhythmias, and determination of the ischemic area in conscious rats. Journal of Pharmacological Methods 1983; 9(3): 219-230
20. Leonard CE, Hennessy S, Han X, Siscovick DS, Flory JH, Deo R. Pro- and Antiarrhythmic Actions of Sulfonylureas: Mechanistic and Clinical Evidence. Trends Endocrinology Metabolism 2017; 28(8): 561-586.
21. Spinelli W, Sorota S, Siegal M, Hoffman BF. Antiarrhythmic actions of the ATP-regulated K+ current activated by pinacidil. Circulation Research 1991; 68(4): 1127-1137.
22. Friedel HA, Brogden RN. Pinacidil. A review of its pharmacodynamic and pharmacokinetic properties, and therapeutic potential in the treatment of hypertension. Drugs 1990; 39(6): 929-967.
23. Iguchi, K., Saotome, M., Yamashita, K., Ikoma, T., Hasan, P., Maekawa, Y., & Watanabe, Y. The Effects of Pinacidil, an ATP Sensitive K+ Channel Opener on Cardiac Na+/Ca2+ Exchanger Function in Guinea Pig Cardiomyocytes. Biophysical Journal 2019; 116(3): 98a-99a.
24. Tosaki A, Szerdahelyi P, Das DK. Reperfusion-induced arrhythmias and myocardial ion shifts: a pharmacologic interaction between pinacidil and cicletanine in isolated rat hearts. Basic Research Cardiology 1992; 87(4): 366-384.
25. Zhang L, Cao S, Deng S, Yao G, Yu T. Ischemic postconditioning and pinacidil suppress calcium overload in anoxia-reoxygenation cardiomyocytes via down-regulation of the calcium-sensing receptor. Peer Journal 2016; 2612; 1-14.
26. Antzelevitch, C., & Di Diego, J. M. Role of K+ channel activators in cardiac electrophysiology and arrhythmias. Circulation 1992; 85(4): 1627-1629
27. Frommeyer G, Garthmann J, Ellermann C, Dechering DG, Kochhäuser S, Reinke F, Köbe J, Wasmer K, Eckardt L. Broad antiarrhythmic effect of mexiletine in different arrhythmia models. Europace 2018; 20(8): 1375-1381.

28. An MY, Sun K, Li Y, Pan YY, Yin YQ, Kang Y, Sun T, Wu H, Gao WZ, Lou JS. Therapeutic effects of a taurine-magnesium coordination compound on experimental models of type 2 short QT syndrome. Acta Pharmacologica Sinica 2018; 39(3): 382-392.
29. Cole WC, McPherson CD, Sontag D. ATP-regulated K+ channels protect the myocardium against ischemia/reperfusion damage. Circulation Research 1991; 69(3):571-81.
30. Di Diego, J. M., & Antzelevitch, C. Pinacidil-induced electrical heterogeneity and extrasystolic activity in canine ventricular tissues. Does activation of ATP-regulated potassium current promote phase 2 reentry? Circulation 1993; 88(3): 1177-1189
31. Yan GX, Antzelevitch C. Cellular basis for the Brugada syndrome and other mechanisms of arrhythmogenesis associated with ST-segment elevation. Circulation 1999;100(15):1660-1666.
32. Ichiro Watanabe and Leonard S. Gettes. Effects of Pinacidil on ST-T Wave Alternans During Acute Myocardial Ischemia in the In-situ Pig Heart. J. Nihon University. Medical Association 2017; 76 (6): 273–279
33. Aravind, S. R., Mittal, S., Venkatraman, S., Deka, N., Parmar, G., Amarnath, S., & Mohan, V. Cardiovascular Profile of Modern Sulfonylureas: Focus on Glimepiride. Journal of the Association of Physicians of India 2019; 67: 17
34. Leonard CE, Brensinger CM, Aquilante CL, Bilker WB, Boudreau DM, Deo R, Flory JH, Gagne JJ, Mangaali MJ, Hennessy S. Comparative Safety of Sulfonylureas and the Risk of Sudden Cardiac Arrest and Ventricular Arrhythmia. Diabetes Care 2018; 41(4): 713-722.
35. Hausenloy, D. J., Wynne, A. M., Mocanu, M. M., & Yellon, D. M. Glimepiride treatment facilitates ischemic preconditioning in the diabetic heart. Journal of cardiovascular pharmacology and therapeutics 2013; 18(3): 263-269
36. Vajda S, Baczkó I, Leprán I. Selective cardiac plasma-membrane K(ATP) channel inhibition is defibrillatory and improves survival during acute myocardial ischemia and reperfusion. European Journal of Pharmacology 2007; 577(1-3): 115-23.
37. Végh A, Papp Hemodynamic and other effects of sulphonylurea drugs on the heart. Diabetes Research and Clinical Practice 1996; 31 Suppl: S43-53.
38. Hayashi, H., Terada, H., & McDonald T. F. Electrical heterogeneity and conduction block in reoxygenated guinea pig papillary muscles. Japanese heart journal 1996; 37(3): 383-391