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

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Objective(s): Electrical inhomogeneity between ischemic and non ischemic myocardium is the basis of arrhythmia which occurs following coronary artery occlusion. The leakage of potassium from the ischemic region to the non ischemic region is very effective in the generation of these arrhythmias. The aim of this study is to research the effect of ATP-dependent potassium (K\text{ATP}) channel blocker (glibenclamide) and opener (pinacidil) on ischemia induced arrhythmia in the presence of small and large infarct sizes.

Materials and Methods: In this study Sprague-Dawley male rats of 8-9 months of age were used. Ischemia was produced by the partial ligation of left coronary artery ramus descending (PL) for smaller infarct and complete ligation of this artery (CL) for larger infarct for 30 min. The arrhythmia score which was calculated from the duration and type of arrhythmia was significantly higher in animals which had a larger infarct area than the animals which had a smaller infarct.

Results: Glibenclamide increased the rate of arrhythmia in animals having smaller infarct but not in animals having larger infarct. Pinacidil did not affect the occurrence of arrhythmia in either group. There was a significant difference in the infarct size and risk of infarct zone between animals which had small and large infarct sizes. The effect of glibenclamide and pinacidil on the arrhythmias differed depend on decrease of infarct size.

Conclusion: Glibenclamide is not effective to decrease ischemia induced arrhythmia in the presence of small and pinacidil in large ischemic zone.

Introduction

Cardiovascular diseases are the main causes of sudden death in both men and women. About 50% of cardiovascular mortality is caused by coronary artery disease (CAD) (1). The major reason for death related to CAD is the lethal arrhythmias that appear after the coronary occlusion. It is known that one of the factors underlying these arrhythmias is the potassium leakage from the ischemic cells (2-4). The blockage of potassium leakage from ischemic cells can be achieved using an ATP-dependent potassium (K\text{ATP}) channel blocker. The antiarrhythmic effects of K\text{ATP} channel blockers have been demonstrated in some studies (5-10). But other studies showed that K\text{ATP} channel blockers are proarrhythmic in male animals during reperfusion (11, 12). On the other hand, K\text{ATP} channel openers have also a proarrhythmic effect (13, 14). The main reason for this proarrhythmic effect depends on the reentrant arrhythmia caused by the shortening of the action potential duration in myocardial cells (15). However, some researchers reported the antiarrhythmic effect of K\text{ATP} channel openers (8, 16 - 18). This different effect may depend on changes in heterogeneity in action potential duration in the ischemic myocardium. This type of heterogeneity is greater between ischemic and non ischemic myocardium. Glibenclamide or pinacidil may decrease the heterogeneity and the likelihood of arrhythmia. The infarct size may be important in the effect of K\text{ATP} channel modulators on decreasing electrical inhomogeneity and arrhythmia. The arrhythmia and the rate of death in experimentally produced myocardial infarction have been observed more in animals which have a larger infarct size (19).

Since glibenclamide closes the ischemic myocar-
diadium to the normal myocardium, it may be effective in decreasing electrical inhomogeneity and arrhythmia. There was no study found showing the effect of $K_{\text{ATP}}$ channel modulators on arrhythmia in relation to different infarct sizes. In this study, it is hypothesized that glibenclamide decrease electrical inhomogeneity and the occurrence of arrhythmia in the presence of small and pinacidil large infarct sizes.

Materials and Methods

**Animals**

In this study, male Sprague-Dawley rats 46 (8-9 months old) were used. The animals were allowed to drink tap water *ad libitum* and were fed with commercial rat food pellet. Two main groups were designed; the first group was designated as partial ligation (PL), and the second one was designated as complete ligation (CL). Each main group had three subgroups: control, glibenclamide, pinacidil. All study protocols were approved by the Ethical Committee of Abant Izzet Baysal University, Bolu, Turkey.

**Operation**

Animals were anesthetized with thiopental sodium (100 mg/kg). The trachea was cannulated for artificial respiration. The catheter for carotid cannulation was filled with heparin (25000 IU/100 ml) diluted 1/10 with saline and inserted into the left carotid artery for the measurement of blood pressure with a blood pressure transducer (SS 13 L, Biopac Systems, Santa Barbara, CA, USA). The chest was opened in the fourth intercostal space. The pericardium was removed and the heart was exposed.

A loose loop of 5-0 atraumatic silk was quickly placed below the side branch of the left coronary artery ramus descending at the level of the left atrial appendage for small infarct, and around the branching point of the left coronary artery from ramus circumflexus from which only the left arterial descending branch was ligated. Then, the heart was set back in its place and artificial respiration was started using an animal respirator at 60 strokes/min, 9 ml/kg (Ugo Basile Rodent Ventilator, Italy). Subcutaneous needle electrodes were placed under the skin to record the electrocardiogram (ECG). Then, the animals were allowed to stabilize for 5 min. The ligation was made by tightening the silk by producing a bowknot to induce myocardial ischemia for 30 min. Arterial blood pressure and bipolar ECG were recorded over the 30 min of coronary ligation (Biopac Systems, Santa Barbara, CA, USA). At the end of the experiment, live animals were heparinized (500 IU/kg) and the heart was excised. Hearts were perfused with 10 ml of isotonic NaCl solution and then 2 ml of ethanol (96%) for demarcation of the occluded and nonoccluded myocardium (20). After the perfusion, the nonperfused area that remained red was separated from the well perfused area that appeared white in color. The nonperfused myocardium was cut and weighed. The percentage of this area in respect to the total weight of the ventricle was determined. After that, the non-perfused myocardium was cut into about six segments by razor blade. These segments were put in the solution prepared with nitro blue tetrazolium (NBT) (Sigma-Aldrich CO) about 3 min for staining. The non ischemic region was stained with NBT but the ischemic area was not stained. The color of non ischemic region was light in pallor whereas the infarcted region was red in color.

The infarct region was separated from the noninfarcted region by razor blade and weighed. Finally, the percentage of the risk of arrhythmia to the infarct area (nonperfused area/all of the heart) and infarct area (infarct area/all of the heart) were calculated.

**Preparation of drugs**

Pinacidil (Sigma-Aldrich CO) was dissolved in ethyl alcohol (96%) and then mixed with isotonic solution and it was given in 0.1 mg/0.2 ml/kg doses intravenously from a coccyeal tail vein 10 min before coronary ligation. Glibenclamide (Sigma-Aldrich CO) was dissolved in dimethylsulfoxide and then mixed with ethanol in the same ratio (1/1).

Glibenclamide was given in 5 mg/0.2 ml/kg doses intraperitoneally 20 min before coronary ligation. In the control group, instead of the pinacidil and glibenclamide, the same value of solvent 0.2 ml/kg was applied in both regions. The doses of both drugs used in this study were chosen according to the studies performed previously (21). Since pinacidil was also a hypotensive agent, larger doses usually lead to the dead of animals due to bradycardia and cardiac arrest. Otherwise glybenclamide is more effective in 5 mg/kg dose in intraperitoneal injection. Lower doses of glibenclamide which were tested in previous study were found to be ineffective on ischemia induced arrhythmia (10).

**The evaluation of the arrhythmias**

The heart rate and blood pressure were determined from recorded ECG before and at 0.5, 1, 10, and 30 min during the 30 min of coronary ligation. The incidence and the total length of arrhythmia were calculated from the recorded ECG. The incidence of arrhythmias was analyzed in accordance with the Lambeth Conventions (22), as ventricular fibrillation (VF), ventricular tachycardia (VT), and other types of arrhythmias including ventricular premature contractions (VPC) and ventricular bigeminy. An arrhythmia score was given according to the incidence and duration of arrhythmias for each animal as follows; 0= No arrhythmias; 1= <10 sec VF or other arrhythmias; 2= 11-30 sec VT or other arrhythmias; 3= 31-90 sec VT or other arrhythmias; 4= 91-180 sec VT or other arrhythmias and/or<10 sec reversible VF; 5=>180 sec VT or other arrhythmias and/or >10 sec reversible
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Table 1. Mean heart rate and blood pressure before and following minutes of coronary ligation

| Coronary ligation | Heart rate (beats/minute) | Blood pressure (mmHg) |
|-------------------|---------------------------|-----------------------|
|                   | Basal 0.5× min 1× min 10× min 30× min Basal 0.5× min 1× min 10× min 30× min | |
| Control PL        | 369 ± 20 368 ± 20 355 ± 23 346 ± 17 | 317 ± 11 104 ± 4 | 91 ± 6 90 ± 8 91 ± 9 74 ± 7 |
| GL PL             | 347 ± 15 352 ± 13 351 ± 18 347 ± 16 | 354 ± 16̅ 111 ± 5 | 86 ± 6 72 ± 9 97 ± 8 98 ± 6̅ |
| Pin PL            | 350 ± 9 349 ± 9 351 ± 6 348 ± 10 | 355 ± 7̅ 119 ± 4̅ | 92 ± 5 93 ± 5 104 ± 6 100 ± 7̅ |
| Control CL        | 317 ± 12 369 ± 12 363 ± 11 379 ± 15 | 349 ± 14 104 ± 5 | 70 ± 4 78 ± 4 92 ± 6 95 ± 7̅ |
| GL CL             | 345 ± 12 329 ± 12̅ 341 ± 12 328 ± 14̅ | 323 ± 14 100 ± 6 | 70 ± 5 68 ± 6 69 ± 6̅ 84 ± 7 |
| Pin CL            | 390 ± 7 366 ± 7 381 ± 9 364 ± 10 | 346 ± 8 98 ± 5 | 61 ± 7 60 ± 8 70 ± 7̅ 54 ± 7̅ |

*P-value < 0.05; according to control PL group. †P-value < 0.05; according to control CL group. Basal; one min before coronary ligation and at 0.5×, 1×, 10×, and 30× min of coronary ligation. CL: complete ligation of left coronary artery ramus descending. PL: partial ligation of left coronary artery ramus descending. Gli: glibenclamide, Pin: pinacidil. Values represent mean ± SEM

VF; 6= irreversible VF (21).

Statistical analyses

All data are expressed as mean±SEM. The differences between groups were calculated using One-way ANOVA and LSD post-hoc test. The survival rate and the incidence of arrhythmia were compared by Chi-square test or Fisher exact test, two tailed.

Results

Changes in the form of QRS complex and/or ST segment elevation were observed in all groups following the coronary ligation. All animals survived after 30 min of coronary ligation in the PL group. However, two animals in CL group died due to irreversible fibrillation. Blood pressure significantly decreased immediately after coronary ligation in all groups of animals (P-value < 0.05) (Table 1). However, the decrease in blood pressure following ligation in CL group was more than the PL group.

Arrhythmias started at 2-5 min following coronary ligation and terminated at about 14 min of coronary ligation. Glibenclamide significantly increased the length of the arrhythmic period in CL group in respect to control (P-value < 0.05) (Table 2). But it was not effective on the arrhythmic periods of PL group animals.

The length of ventricular tachycardia, total arrhythmia, and other types of arrhythmia were lower in the PL group in respect to the CL group (P-value < 0.05). Glibenclamide and pinacidil did not affect the incidence of arrhythmias in either group in respect to the corresponding controls (Table 3).

Arrhythmia started earlier in the PL group than the CL group. Pinacidil lengthened the onset of arrhythmia in the PL group but not in the CL group. The effect of both glibenclamide and pinacidil on arrhythmic period was found to be similar in the PL and CL groups. Glibenclamide increased the arrhythmic period significantly in CL group (P-value < 0.05) but non significantly in PL group (P-value = 0.61), but pinacidil non significantly decreased the arrhythmic period in the CL (P-value = 0.46) and PL (P-value = 0.35) groups. As expected, the score of arrhythmia was significantly higher in the CL group than the PL group (P-value < 0.05). Glibenclamide significantly increased the score of arrhythmia in PL group (P-value < 0.05) but the effects of pinacidil were not significant (P-value = 0.21) (Figure 1). Both glibenclamide and pinacidil did not affect the score of arrhythmia in CL group.

The risk of infarct zone and infarct area was larger in the CL group than the PL group (P-value < 0.05). Glibenclamide and pinacidil non significantly increased the infarct area in the PL group (P-value = 0.24) but they were not effective in the CL group, (Table 4).

Risk of infarct area% = weight of infarct area × 100/weight of whole ventricular myocardium.

Infarct area% = weight of infarct area × 100/weight of whole ventricular myocardium. *P-value < 0.05; according to control CL group.

Table 2. Duration of arrhythmias occurred in 30 minute coronary ligation

| Groups   | N | Onset of arrhythmia (min) | Arrhythmic period (min) | VF | VT | Other | Total |
|----------|---|---------------------------|-------------------------|----|----|-------|-------|
| Control PL | 7 | 2.18 ± 0.94*             | 13.09 ± 3.40            | 0  | 0.023 ± 0.015* | 0.44 ± 0.18* | 0.46 ± 0.18* |
| GL PL     | 7 | 4.40 ± 1.04              | 14.61 ± 1.18            | 0.026 ± 0.017 | 0.03 ± 0.02 | 1.1 ± 0.25 | 1.15 ± 0.26 |
| Pin PL    | 7 | 4.88 ± 0.91*             | 10.30 ± 1.21            | 0  | 0  | 0.8 ± 0.14 | 0.79 ± 0.14 |
| Control CL | 8 | 5.88 ± 0.57              | 15.44 ± 2.22            | 0  | 1.02 ± 0.44 | 1.68 ± 0.28 | 2.7 ± 0.71 |
| GL CL     | 7 | 3.99 ± 0.94              | 21.57 ± 1.64*           | 0.021 ± 0.017 | 0.48 ± 0.47 | 2.64 ± 0.68 | 3.15 ± 0.79 |
| Pin CL    | 8 | 5.82 ± 0.26              | 13.39 ± 1.71            | 0.06 ± 0.06 | 0.54 ± 0.48 | 2.08 ± 0.38 | 2.68 ± 0.85 |

*P-value < 0.05; according to control CL group. †P-value < 0.05; according to control PL group. N: the number of animals at the end of 30 min of ligation. VF: ventricular fibrillation, VT: ventricular tachycardia, Other: ventricular extra beats and bigeminy CL: complete ligation of left coronary artery ramus descending. PL: partial ligation of left coronary artery ramus descending. Gli: glibenclamide, Pin: pinacidil. Values represent mean ± SEM

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In a previous recent study (26), the arrhythmia in animals with especially small infarct size was thought to decrease arrhythmia in this group. But pinacidil was not found to be effective on the arrhythmia in animals having larger infarct size.

Table 4. The percentage of risk of infarct zone and infarct area measured at the end of 30 minutes coronary ligation

| Groups | N  | Risk of infarct zone % | Infarct area % |
|--------|----|------------------------|----------------|
| Control CL | 7 | 29.42 ± 1.86* | 7.85 ± 2.20* |
| Gli. PL | 7 | 33.00 ± 2.00 | 10.28 ± 1.71 |
| Pin. PL | 7 | 30.42 ± 2.18 | 10.28 ± 0.99 |
| Control CL | 10 | 48.01 ± 1.45 | 20.99 ± 1.41 |
| Gli. CL | 7 | 45.87 ± 1.16 | 20.70 ± 1.13 |
| Pin. CL | 8 | 5.168 ± 1.52 | 22.08 ± 0.65 |

*P-value <0.05; according to control CL group. N: the number of animals at the end of 30 min of ligation CL: complete ligation of left coronary artery ramus descending, PL: partial ligation of left coronary artery ramus descending, Gli: glibenclamide, Pin: pinacidil. Values represent mean ± SEM.
Glibenclamide did not affect the arrhythmia following complete ligation which was similar with the findings of our previous study (27). In addition, pinacidil did not have significant effect on arrhythmias in either animal with small or large infarcts produced by 30 min of ligation. In contrast to the most of the studies, pinacidil did not have proarrhythmic (8, 17) or antiarrhythmic effects (13) following ligation. However, it was seen that pinacidil increased the severity of arrhythmia non significantly in animals with smaller infarcts size.

Glibenclamide and pinacidil increased the infarct area non significantly in animal having partial coronary ligation, but they did not have any effect on the ischemia induced arrhythmia in animals which had complete ligation. Arrhythmia started earlier in group with smaller infarct size which may be caused by lower reflex activation of sympathetic system. The fall of the blood pressure immediately following ligation was lower in the PL group than the CL group which may be attributable to the short lasting sympathetic overdrive suppression in the PL group. Pinacidil did not have any effect on the arrhythmic period in either group of animals. Since no comparable study was found in the literature, the results related with the arrhythmic period could not be compared.

Conclusion
As a result, it seems that the effect of glibenclamide and pinacidil on the severity of arrhythmia changes depend on the change of the ischemic zone size. Glibenclamide increased arrhythmia in the presence of smaller risk of infarct zone but was ineffective in the presence of larger infarct size. Pinacidil did not change the severity of arrhythmia depend on the changes in ischemic zone, but it non significantly increased the total arrhythmia in the presence of smaller ischemic zone. Underlying cellular mechanisms of different effect of glibenclamide and pinacidil on the ischemia induced arrhythmia in small and large infarct size are not explained by the result of present study. Further electrophysiologic researches are required in this case. Since the electrical inhomogeneity is important in the generation of ischemia induced arrhythmias, more drugs or procedures should be researched to decrease electrical inhomogeneity.

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