

Effects of Metformin on QT and QTc Interval Dispersion of Diabetic Rats

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Summary

Background: Several drugs can cause prolonged QT interval, as well as prolonged QT dispersion (QTd) in electrocardiographic (EKG) recordings. QTd may be a potentially sensitive marker of increased risk of cardiac arrhythmias and sudden cardiac death. Metformin is an effective antihyperglycemic agent used in the treatment of diabetes. However, studies have correlated dose-dependent effects of metformin on glycemia and cardiovascular risk markers.

Objective: To evaluate the dose-response effects of metformin on QT and QTd of diabetic rats.

Methods: Male Wistar rats were distributed in five groups: non-treated control (C), non-treated diabetics (D), diabetics treated with metformin at the doses of 3.5, 30 and 74 $\mu\text{g}/\text{kg}/\text{bw}$ (DM 3.5, DM 30 and DM 74). Diabetes was induced by an alloxan injection (40 mg/kg, IV). EKG was recorded (days 1, 15 and 30) using four electrodes inserted into the subcutaneous layer of the paws. Both RR and QT intervals were measured, and then corrected QT and QT dispersion values were calculated.

Results: The DM 3.5 and DM 30 groups showed a significant reduction of glycemia ($p < 0.05$) when compared with the high dose (DM 74). Rats of the DM 74 group presented prolonged QTc, QTd and QTcd intervals, whereas rats of the DM 3.5 and DM 30 groups presented less prolonged intervals.

Conclusion: Metformin at high doses provided greater dispersion of the QT interval probably because of the increased ventricular repolarization inhomogeneity, whereas at low doses decreased QT intervals were observed in diabetic rats. (Arq Bras Cardiol 2008; 90(4): 232-238)

Key words: Diabetes mellitus, experimental; electrocardiography; metformin.

Introduction

Diabetes is a metabolic disorder characterized by increased blood glucose resulting from alterations in protein, lipid and carbohydrate metabolism due to impaired secretion of or peripheral resistance to insulin. These alterations increase the tendency to the development of cardiovascular diseases and neuropathies, which represent one of the major causes of death of patients^{1,2}. Alterations in carbohydrate and lipid metabolism as well as electrolytic changes in blood calcium and potassium may cause modifications in the cardiovascular structure and physiology which, in turn, lead to alterations in electrocardiographic recording^{3,4}.

Electrocardiogram (ECG) is one of the most important methods in the diagnosis of heart diseases⁵. Depending on the extent and areas damaged, the propagation of action potentials in the heart muscle changes ECG waves and their respective intervals.

Among the main changes observed is the QT interval prolongation, also known as long QT syndrome (LQTS).

LQTS may be congenital – when abnormalities in sodium or potassium channels occur, or acquired – resulting from the use of medications, electrical abnormalities or metabolic disorders. The QT interval corresponds to the time necessary for the complete ventricular electrical excitation and recovery and is, therefore, the measurement of the duration of the “electrical” systole, which includes ventricular depolarization and repolarization, that is, the total duration of the ventricular electrical activity⁶. The QT interval varies inversely with heart rate (HR), so that the higher the HR, the shorter the QT and vice versa. Accordingly, it should be corrected for HR, thus generating the QTc, which is preferably used⁷. Other parameters to be assessed are QT dispersion and QTc dispersion (QTd and QTcd, respectively). This dispersion would result from inhomogeneity of repolarization times, and is defined as the difference between the maximum and minimum QT in the twelve electrocardiographic leads⁸. QT dispersion is a marker of arrhythmogenesis, and its analysis is a noninvasive method for the detection of ventricular repolarization heterogeneity⁹. Additionally, some studies associate increased QTd intervals with a higher risk of sudden death^{10,11} and as a prognostic index in heart failure and hypertrophic cardiomyopathy^{8,12}. In diabetic individuals, increased QT interval is considered a precursor of increased risk of sudden death¹³.

Depending on their dosages, certain medications may prolong the duration of the ventricular action potential and

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the QT interval by means of different ionic mechanisms.

The biguanide metformin is an antihyperglycemic agent used to reduce blood glucose and consequently to improve the metabolic profile. It reduces blood glucose levels by inhibiting gluconeogenesis and in the presence of insulin it stimulates peripheral glucose uptake by the tissues – mainly skeletal muscles – and decreases absorption of glucose from the gastrointestinal tract. Its direct effect on β cells is still debatable. Metformin does not cause hypoglycemia, but rather reduces glycosylated hemoglobin levels and improves the lipid profile and fibrinolytic activity^{14,15}.

Despite the beneficial effects of metformin, studies have demonstrated that its use at high doses does not decrease hyperglycemia and increases the cardiovascular risks¹⁶⁻²⁰.

Gastrointestinal disorders such as diarrhea are common, and the intestinal absorption of group B vitamins, especially folate, is impaired during chronic therapy. Frequently, this deficiency increases plasma homocysteine levels, which accelerate the progression of vascular disease due to adverse effects on blood vessels, clotting factors, and endothelium. One study found an association between high homocysteine levels and the overall mortality in patients with atherogenic disease¹⁵.

In some circumstances, metformin may lead to lactic acidosis, especially in patients with clinical conditions that predisposed to this complication, such as heart failure or myocardial infarction¹⁵.

Additionally, preliminary results (unpublished) showed that high metformin doses such as those recommended for human patients promoted a great increase in cardiac glycogen in alloxanized rats, whereas at low doses this increase was milder. In diabetic patients, the increased glycogen concentration in the ventricles is apparently associated with the severity of diabetes, and makes the heart more susceptible to the effects of ischemia^{21,22}, in addition to leading to cardiac malfunctioning, since its accumulation in the conductive tissue is the cause of sinus and atrioventricular node dysfunction²³.

Based on these findings, we investigated the effects of low and high metformin doses on blood glucose and on the QT interval, as well as on QTc, QTd, and QTcd of alloxan diabetic rats.

Methods

Animals

Male albino Wistar rats aged 10 weeks were supplied by Unicamp's vivarium to be used in this study. They were kept in the vivarium of the Department of Physiology and Biophysics for adaptation for two weeks. The rats were fed on feed (Purina rodent) and had ad libitum access to water in a photoperiodic cycle of 12 h light and 12 h dark, at $22 \pm 2^\circ$ C. The experimental protocol was approved by the Ethics Committee on Animal Experimentation of Unicamp under number 262-1.

Induction of diabetes

Before induction of diabetes, the rats were fasted for 24 hours with free access to water. After being anesthetized with sodium pentobarbital (40 mg/kg of body weight), the rats were injected with alloxan in saline (40 mg/kg/bw, IV, pH 4.5,

Sigma™)^{24,25}. Established diabetes was verified by the presence of glucose in urine the next day.

Treatment with metformin and groups

After induction of diabetes, the treatment was started. The rats in the groups treated received metformin (Lipha, Lyon, France) diluted in drinking water (3.5, 30 and 74 μ g/g/bw) for 30 days, thus comprising the following groups: control (C; n = 5); diabetes (D; n = 6); diabetes metformin [3,5 μ g/g/bw] (DM3.5; n = 8); diabetes metformin [30 μ g/g/bw] (DM30; n = 7); and diabetes metformin [74 μ g/g/bw] (DM74; n = 8).

Electrocardiogram

Anesthetized rats (sodium pentobarbital, 40 mg/kg/bw) were kept in the supine position with spontaneous breathing for ECG recording. The electrodes were connected to the computer channels (Heart Ware System), and six standard waves were recorded (I, II, III, aVR, aVL and aVF) with a 2N sensitivity at a 50-mm/second speed. The QT interval was measured in three consecutive beats from the beginning of the QRS-complex to the point of return of the T wave to the isoelectric line, defined as the TP segment²⁶. QTd was calculated in absolute values, by subtracting the shortest QT interval from the longest (QT = QT max – Qt min). This value was converted into a percentage (%QTd) by correcting the QTd for the shortest QT interval and multiplying this value by 100 (%QTd = Qt max – QT min/QT min x 100). The QT interval was corrected for the heart rate using Bazett's formula (QTc = QT/ $\sqrt{R-R}$) and the QTc interval dispersion was further calculated by subtracting the minimum QTc interval from the maximum QTc interval (QTcd = QTcmax - QTcmin). The percentage QTcd was also calculated (%QTcd = Qtcmax - QTcmin/ Qtcmin x 100). The analyses were made by a single observer blinded to the treatment the animals received, so as to minimize divergences in the dispersion measurement.

Determination of plasma glucose, Na⁺, K⁺ and Ca²⁺

Plasma glucose was determined with the glucose oxidase method, using a commercial kit (Laborlab, Sao Paulo, Brazil). Na⁺ and K⁺ were determined using the selective ion electrode method (Roche™). Total serum calcium was measured using the colorimetric method with cresolphaleine (o-cresolphaleine complexone – Roche™)²⁷, with an automated analyzer (Hitachi™).

Statistics

The results were expressed as mean \pm MSD. Data were evaluated using the analysis of variance for two factors, followed by Tukey's test. Significant values were considered those whose differences were lower than 5%.

Results

Effects of metformin on blood glucose

Blood glucose levels did not differ among the rats of all groups at the beginning of the experimental period (day 1).

At the end of the experiment, similar levels were observed in rats of the control group.

Administration of alloxan induced severe hyperglycemia on day 15, which was maintained until day 30 in the rats of group D (563 ± 61 mg/dl, $p < 0.001$; compared with blood glucose prior to alloxan administration). Rats that received daily metformin doses of 3.5 (DME.5) and 30 (DM30) mg/kg showed lower blood glucose levels on days 15 and 30 (381 ± 37 and 360 ± 14 , respectively) of treatment when compared with rats of the control diabetic group. The administration of metformin at 74 mg/kg marginally reduced blood glucose after day 30 in comparison with control diabetic rats ($p > 0.05$) (Fig.1).

Effects of metformin on ECG

Figure 2 (A, B, C and D) shows that the QT (2A) and QTc (B) intervals, as well as QTd (2C) and QTcd (2D), were similar at the beginning of the experimental period. In diabetic rats, these intervals increased after 15 days of the administration of alloxan and remained similar 30 days later. On days 15 and 30, the QT interval of diabetic rats treated with 3.5 and 30 mg/kg was similar to that observed in the non-diabetic control group, but was significantly longer in rats treated with metformin at 74 mg/kg. The QTc interval of rats treated with lower metformin doses (3.5 and 30 mg/kg) was longer than that observed in rats of the control group (non-diabetic) 15 and 30 days after the administration of alloxan. However, it was significantly shorter than the values observed in diabetics (non-treated) and diabetics treated with metformin at high doses (74 mg/kg). Only metformin at lower doses (3.5 and 30 mg/kg) decreased QT and QTc dispersion when compared with the group of non-treated diabetics. However, these differences were more evident after 30 days of treatment.

Effects of metformin treatment on serum ion concentration

Table 1 shows a decrease in serum K^+ concentration of diabetic rats (non-treated) in comparison with the control group (non-diabetic).

It also shows an increase in Ca^{2+} concentration only in the group treated with metformin at high doses. No alterations were observed in the other parameters.

Discussion

Type-2 diabetes is characterized by alterations in glucose and lipid metabolism, partly due to resistance to insulin action in peripheral tissues. If untreated, it may lead to several complications such as increased cardiovascular risk^{1,2}.

The biguanide metformin is an antihyperglycemic agent used in the treatment of type-2 diabetics. However, the action mechanism of metformin is still unclear.

Studies have shown that metformin activates AMP activated protein kinase (AMPK) in liver cells and skeletal muscle of rats²⁸. Recent studies indicate that AMPK plays a role in the regulation of glucose and lipid metabolism. AMPK is an enzyme that promotes energy availability and is activated by skeletal muscle contraction and during myocardial ischemia; it is involved in the stimulation of glucose transport and fatty acid oxidation.

In the liver, AMPK activation results in increased fatty acid oxidation and decreased production of glucose, cholesterol and triglyceride²⁹. This effect contributes to a reduction in blood glucose and improvement of the metabolic profile, and leads to increased glycogen in the liver and muscles, in addition to promoting benefits to blood lipid profile^{30,31}.

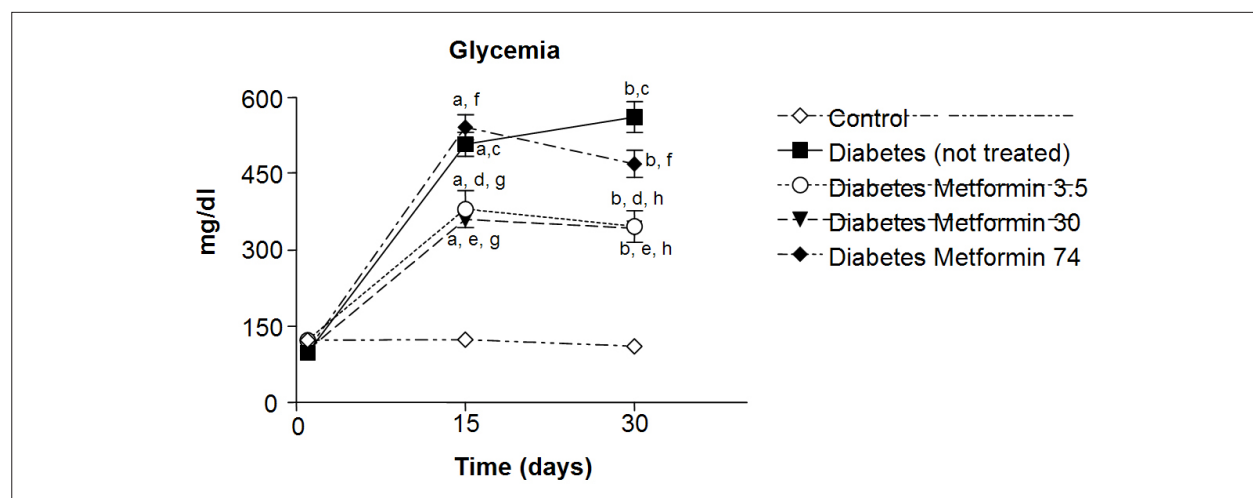


Figure 1 - Changes in glycemia (mg/dl) of rats after alloxan induction and after 15 and 30 days of treatment with metformin at low (3.5 μ g/g/bw), medium (30 μ g/g/bw) and high doses (74 μ g/g/bw). Data are expressed as mean \pm MSD. a - significantly different from the control group on day 15 of the experimental period ($p < 0.05$); b - significantly different from the control group on day 30 of the experimental period ($p < 0.05$); c - significantly different from the diabetes group (not treated) on day 1 of the experimental period ($p < 0.05$); d - significantly different from the diabetes metformin 3.5 group on day 1 of the experimental period ($p < 0.05$); e - significantly different from the diabetes metformin 30 group on day 1 of the experimental period ($p < 0.05$); f - significantly different from the diabetes metformin 74 group on day 1 of the experimental period ($p < 0.05$); g - significantly different from the diabetes group (not treated) on day 15 of the experimental period ($p < 0.05$); h - significantly different from the diabetes group (not treated) on day 30 of the experimental period ($p < 0.05$).

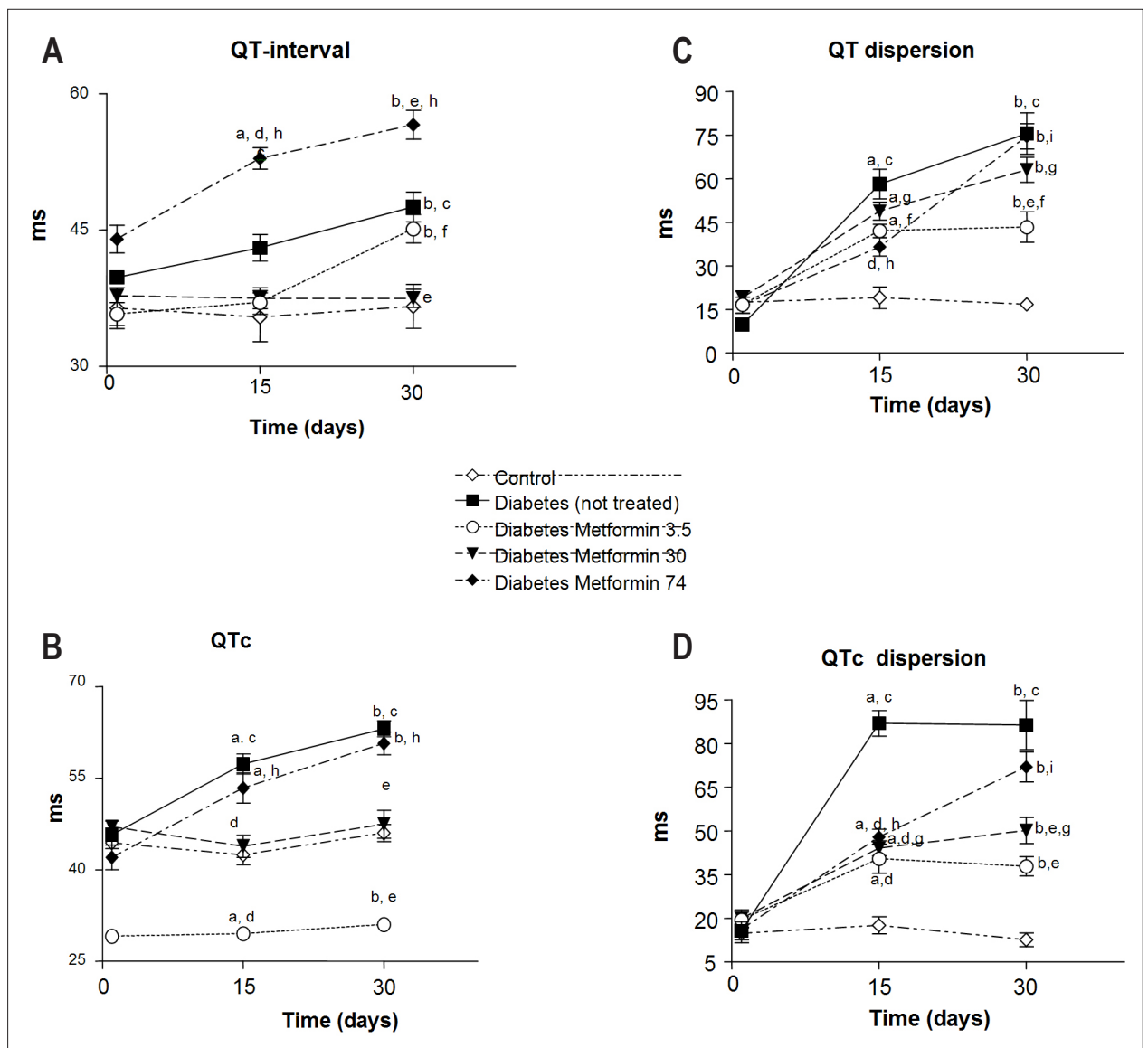


Figure 2 A, B, C, D - Changes in QT, QTc, QTd, QTcd intervals (ms) in rats after alloxan induction and after 15 and 30 days of treatment with metformin at low (3.5 $\mu\text{g/g/bw}$), medium (30 $\mu\text{g/g/bw}$) and high doses (74 $\mu\text{g/g/bw}$). Data are expressed as mean \pm MSD. a - significantly different from the control group on day 15 of the experimental period ($p < 0.05$); b - significantly different from the control group on day 30 of the experimental period ($p < 0.05$); c - significantly different from the diabetes group (not treated) on day 1 of the experimental period ($p < 0.05$); d - significantly different from the diabetes group (not treated) on day 1 of the experimental period ($p < 0.05$); e - significantly different from the diabetes group (not treated) on day 30 of the experimental period ($p < 0.05$); f - significantly different from the diabetes metformin 3.5 group on day 1 of the experimental period ($p < 0.05$); g - significantly different from the diabetes metformin 30 group on day 1 of the experimental period ($p < 0.05$); h - significantly different from the diabetes metformin 74 group on day 30 of the experimental period ($p < 0.05$); i - significantly different from the diabetes metformin 74 group on day 15 of the experimental period ($p < 0.05$).

However, other studies demonstrated that high-dose metformin did not reduce hyperglycemia in diabetic animals, but could increase the cardiovascular risks¹⁶⁻²⁰.

Our results (Figure 1) show that metformin at a high dose equivalent to that recommended for diabetic patients (74 $\mu\text{g/g/bw}$) did not reduce blood glucose in diabetic rats, whereas at low (3.5 $\mu\text{g/g/bw}$) and medium doses (30 $\mu\text{g/g/bw}$), it significantly reduced blood glucose in comparison with untreated diabetic rats. However, the blood glucose reduction induced by low doses of metformin was not enough

to normalize blood glucose levels.

The inefficacy of high doses of metformin in reducing blood glucose may have been caused by decreased β -cell responsiveness to glucose. An in vitro study with β cells showed that metformin at high doses (1 mM) and prolonged exposure (24 hours) activated AMPK and thus partially inhibited the synthesis and release of insulin in β cells, which caused the reduction of their glucose responsiveness³².

Additionally, metformin at therapeutic doses was observed to stimulate the tyrosine kinase activity of the intracellular

Table 1 - Effects of the treatment with metformin at low doses (3.5 µg/g/bw), at medium doses (30 µg/g/bw) and at high doses (74 µg/g/bw) on serum potassium (mEq/l), sodium (mEq/l) and calcium (mg/dl) concentrations. Data are expressed as mean ± MSD

Groups	Sodium	Potassium	Calcium
Control	140 ± 1.08	4.80 ± 0.06	7.05 ± 0.33
Diabetes	127 ± 2.4	4.00 ± 0.11 a	6.80 ± 0.18
DM 3.5	136 ± 2.8	4.73 ± 0.03	7.10 ± 0.30
DM 30	137 ± 1	4.92 ± 0.08	6.64 ± 0.52
DM 74	134 ± 1.4	4.80 ± 0.10	8.76 ± 0.30 a, b, c, d

a - significantly different from the control group (n = 4) on day 30 of the experimental period (p < 0.05); b - significantly different from the diabetes group (non-treated) (n = 4) on day 30 of the experimental period (p < 0.05); c - significantly different from the diabetes metformin 3.5 group (n = 5) on day 30 of the experimental period (p < 0.05); d - significantly different from the diabetes metformin 30 group (n = 5) on day 30 of the experimental period (p < 0.05).

portion of the β subunit of its receptor. However, high doses of metformin inhibited tyrosine kinase activity and, consequently, insulin action³³.

Diabetes, per se, causes changes in MAP kinase and in glucose transporters. The enhancement of K⁺ currents in ventricular cells depends on the activation of MAP kinase, which reflects the synthesis of new channels. The decrease of insulin in diabetics probably leads to a reduction in K⁺ currents in the epicardium, thus causing increased duration of the cardiac action potential³⁴.

The alterations promoted in carbohydrate and lipid metabolism and in electrolyte concentrations may cause changes in the cardiovascular physiology and structure, which can be recorded in the electrocardiogram. One of the most common problems detected in diabetics is prolongation of the QT interval, as well as of QTc, QTd and QTcd. This is due to a late depolarization and/or repolarization of the cardiac action potential, thus leading to an increased risk of arrhythmias and sudden death³⁵.

Although the QT interval is used as a noninvasive method to identify the risk of developing malignant ventricular arrhythmias and sudden death, flaws are actually expected in the system proposed. When the equation QT max – QT min is used, a 12-lead ECG with a well-defined T-wave in each lead should ideally be obtained³⁶. This was a limitation in our study because only six leads can be recorded in rats according to the methodology used by other authors³⁷. Additionally, in several situations it was impossible to detect the end of the T-wave. In this circumstance, the methodology indicates that excluding this interval from the analysis is the most proper thing to do³⁶, as we did in our results. However, despite these limitations, some measures were taken to minimize the discrepancies in the dispersion measurement. The same electrode was used in all electrocardiographic recordings; the site where it was placed was also standardized. The ECG was always performed in the morning so as to lessen the possible effects of the circadian rhythm. Additionally, one single observer analyzed the QT interval in three consecutive beats, in order to decrease counting errors²⁶. After determination of the values,

no statistically significant difference was found between the results calculated.

Thus, despite the limitations of the study, the QTc, QTd and QTcd intervals increased in alloxan diabetic rats, as shown in Figure 2 (B, C and D).

The causes of the increased intervals are unknown. However, this alteration observed in the electrocardiogram may be explained in function of polyuria, which is the most common clinical manifestation of diabetes mellitus³⁸. Polyuria observed in diabetic patients is traditionally attributed to the osmotic diuresis secondary to increased blood glucose (glycosuria)³⁷. The excessive urinary loss causes a reduction of potassium³⁹ which can be responsible for the increased membrane repolarization period⁴⁰, with a consequent increase in the QT interval. The reduction in serum K concentration observed in rats of the diabetes group is shown in Table 1.

The increased QT interval dispersion may also result from alterations in voltage-gated K⁺ channels⁴¹. In fact, diabetes may change the magnitude of K⁺ channels involved in the cardiomyocyte membrane repolarization process³⁴, thus affecting the cardiac tissue.

Treatment of diabetic rats with high doses of metformin (74 µg/g) caused an increase in QT, QTd, QTc, and QTcd on days 15 and 30 of the experimental period (Figure 2 A-D).

This increase may result from the hypercalcemia observed in this group (Table 1), since increased calcium concentrations reduce the ventricular conduction velocity and shorten the effective refractory period, and thus may cause arrhythmias⁴².

However, factors that were not evaluated in this study may also have contributed to the increased QT interval. Persistent AMPK activity, as occurs during treatment with metformin at high doses, may promote mutations in PRKAG2, the gene for γ2 regulatory subunit of AMP-activated protein kinase. This mutation may increase glucose uptake by stimulating translocation of the glucose transporter GLUT-4 to the plasma membrane and increase hexokinase activity, thus leading to glycogen accumulation, which may cause sinus node and atrioventricular node dysfunction^{22,24}.

Additionally, AMPK activation also promotes a dose-dependent increase in glycolysis and fatty acid oxidation in cardiomyocytes. Increased fatty acid oxidation inhibits glucose oxidation, and the acetyl-coA produced may inhibit the pyruvate-dehydrogenase complex, thus limiting pyruvate oxidation. These events lead to increased glycolysis and, in this situation, the protons and lactate produced accumulate and provoke a decrease in pH, intracellular calcium overload and subsequent cell death, thus reducing the cardiac functional capacity¹⁶.

However, metformin at low and medium doses decreased the QT, QTc, QTd, and QTcd intervals, thus suggesting improved ventricular electric conduction. Additionally, serum electrolytes (calcium, potassium and sodium), which are other factors that may affect the electric conductivity, did not change in these groups.

Thus, like in other studies, it is questionable whether QTc

dispersion is actually a more accurate and safe measurement than the measurement of QTc alone as a marker of cardiovascular risk, or whether it is merely one more accurate measure to be added to the clinical armamentarium of assessment of the cardiovascular risk. Regardless of this discussion, we conclude that since QTc dispersion measurement is simple and relatively inexpensive, it is important in order to study the influence of drugs on cardiovascular parameters. Because of the limitations of the methodology used in this study, the results and comments presented are based only on data obtained under our experimental conditions, so as not to incur the risk of improper extrapolations.

Conclusion

A significant increase in QT interval dispersion was observed with high metformin doses. Low and medium metformin doses decreased QT interval dispersion.

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Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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Study Association

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