Ischemic Preconditioning During Coronary Angioplasty Is Prevented by Glibenclamide, a Selective ATP-Sensitive K⁺ Channel Blocker

Fabrizio Tomai, MD; Filippo Crea, MD; Achille Gasparidone, MD, MPhil; Francesco Versaci, MD; Ruggero De Paulis, MD; Alfonso Penta de Peppo, MD; Luigi Chiariello, MD; Pier A. Gioffrè, MD

Background Brief episodes of ischemia render the heart more resistant to subsequent ischemia; this phenomenon has been called ischemic preconditioning. In some animal species, myocardial preconditioning appears to be due to activation of ATP-sensitive K⁺ (K\textsubscript{ATP}) channels. The role played by K\textsubscript{ATP} channels in preconditioning in humans remains unknown. The aim of this study was to establish whether glibenclamide, a selective K\textsubscript{ATP} channel blocker, abolishes the ischemic preconditioning observed in humans during coronary angioplasty following repeated balloon inflations.

Methods and Results Twenty consecutive patients undergoing one-vessel coronary angioplasty were randomized to receive 10 mg oral glibenclamide or placebo. Sixty minutes after glibenclamide or placebo administration, patients were given an infusion of 10% dextrose (8 mL/min) to correct glucose plasma levels or, respectively, an infusion of saline at the same infusion rate. Thirty minutes after the beginning of the infusion, both patient groups underwent coronary angioplasty. The mean values (±1 SD) of ST-segment shifts on the surface 12-lead ECG and the intracoronary ECG were measured at the end of the first and second balloon inflations, both 2 minutes long. In glibenclamide-treated patients, the mean ST-segment shift during the second balloon inflation was similar to that observed during the first inflation (23±13 versus 20±8 mm, P=NS), and the severity of cardiac pain was greater (55±21 versus 43±23 mm on a scale of 0 to 100, P<.05). Conversely, in placebo-treated patients the mean ST-segment shift during the second inflation was less than that during the first inflation (9±5 versus 23±13 mm, P<.001), as was the severity of cardiac pain (15±15 versus 42±19 mm, P<.01). Blood glucose levels were significantly reduced 60 minutes after glibenclamide compared with those at baseline (53±9 versus 102±10 mg/100 mL, P<.001) in the glibenclamide group; however, before coronary angioplasty, blood glucose levels increased to 95±19 mg/100 mL, a value similar to that found in placebo group (96±11 mg/100 mL, P=NS).

Conclusions In humans, ischemic preconditioning during brief repeated coronary occlusions is completely abolished by pretreatment with glibenclamide, thus suggesting that it is mainly mediated by K\textsubscript{ATP} channels. (Circulation. 1994;90:700-705.)

Key Words • ATP • potassium channels • angioplasty • glibenclamide

Recent experiments have demonstrated that brief episodes of myocardial ischemia can render the heart more resistant to subsequent ischemic episodes.\textsuperscript{1-6} This phenomenon has been called ischemic preconditioning.\textsuperscript{1} Several studies suggest that preconditioning may be due to the activation of ATP-sensitive K⁺ (K\textsubscript{ATP}) channels.\textsuperscript{7-16} Gross and Auchampach\textsuperscript{10} were the first to show that preconditioning in dogs is mediated by activation of K\textsubscript{ATP} channels as it was prevented by glibenclamide. Furthermore, Cole et al\textsuperscript{8} demonstrated in guinea pigs that blockade of K\textsubscript{ATP} channels with glibenclamide worsens and that opening K\textsubscript{ATP} channels with pinacidil enhances the electrical and mechanical recovery after ischemia and reperfusion. Conversely, Downey's group failed to find a role for K\textsubscript{ATP} channels in rat hearts.\textsuperscript{17} Finally, the role of K\textsubscript{ATP} channels in rabbit is still controversial.\textsuperscript{14,15,18} Thus, preconditioning appears to operate in many species, but its prevailing mechanisms may differ.

Deutsch et al,\textsuperscript{19} Cribier et al,\textsuperscript{20} and we\textsuperscript{21} have recently shown that during coronary angioplasty, the severity of myocardial ischemia during the second balloon inflation is less than that during the first inflation, thus suggesting that ischemic preconditioning may also occur in humans.

To establish whether the reduction of myocardial ischemia previously observed in humans during brief repeated coronary occlusions\textsuperscript{19-21} is due to activation of K\textsubscript{ATP} channels, we used the selective K\textsubscript{ATP} channel blocker glibenclamide\textsuperscript{22} in a selected group of patients undergoing coronary angioplasty.

Methods

Patients We studied 20 consecutive patients undergoing uncomplicated elective coronary angioplasty who had (1) history of chronic stable angina pectoris lasting ≥3 months; (2) isolated obstructive lesion (internal diameter reduction, >70% by visual assessment) in the proximal two thirds of one major epicardial coronary artery; (3) no history of previous myocardial infarction; and (4) no angiographic evidence of coronary collateral vessels (grade 0, according to Rentrop’s classification\textsuperscript{22}). No patient had evidence of left ventricular hypertrophy on the echocardiogram or conduction defects on the ECG that...
could have interfered with the interpretation of ST-segment changes. All patients had normal hepatic and renal function and fasting blood glucose levels. All patients gave written informed consent for participation in the study, which was approved by the Institutional Ethics Committee in October 1992.

Study Protocol

In this single-blind study, patients were randomly allocated to one of two groups. One group consisted of 10 patients (9 men and 1 woman; age range, 38 to 65 years; mean age, 54 years) who received 10 mg oral glibenclamide; the other group consisted of 10 patients (8 men and 2 women; age range, 48 to 67 years; mean age, 57 years) who received placebo. All patients were receiving oral aspirin (100 mg/d), dilatiazem (60 mg TID), and isosorbide dinitrate (40 mg BID) for ≥48 hours before coronary angioplasty. All patients received the morning dose of treatment before coronary angioplasty, which was performed within the following 4 hours. No patient received sublingual or intravenous nitrates in the last 24 hours before the study or throughout the study. Patients were not premedicated with diazepam or other sedatives. Six minutes after glibenclamide or placebo administration, a continuous infusion of 10% dextrose or saline (8 mL/min), respectively, was started. Thirty minutes after the beginning of the infusion, both patient groups underwent coronary angioplasty. This time interval was chosen as it has previously been demonstrated in volunteers that the peak plasma levels of glibenclamide are achieved between 60 and 120 minutes after the oral administration of glibenclamide.

Coronary angioplasty of the stenosed artery was performed by a standard technique using the femoral approach. After arterial cannulation, an intravenous bolus of 10 000 IU heparin was administered, followed by the intravenous infusion at a rate of 1000 IU/h for 12 hours after the procedure. All procedures were performed with “rapid exchange system” balloon catheters (Rx Streak, Advanced Cardiovascular Systems, Inc) and nonionic contrast medium (iopamiro 370, Bracco spa; 796 mOsmxkg⁻¹). After placement of the guiding catheter and performance of baseline angiography, the guide wire was placed across the lesion. The balloon catheter was then placed within the stenosis, and the balloon was inflated for 2 minutes. After balloon deflation and withdrawal proximal to the lesion with the guide wire remaining across the lesion, a recovery period of ≥5 minutes was allowed to reestablish baseline hemodynamic and ECG conditions. A second balloon inflation for 2 minutes was then performed. In each patient, balloon pressure during the first and second inflations was identical. After the first two inflations, coronary angioplasty was completed on the basis of the specific needs of individual patients.

Assessment of Myocardial Ischemia

Standard surface 12-lead and intracoronary ECGs derived from the coronary guide wire were continuously monitored and simultaneously recorded (Mingograph 7, Siemens) at a paper speed 25 mm/s throughout the study. To avoid electrode interference with fluoroscopic imaging during the angioplasty procedure, radiotranslucent precordial electrodes were used. The ECGs were analyzed by a cardiologist who had no knowledge of the study protocol. At baseline and at the end of the first two inflations, ST-segment shift was measured 80 milliseconds after the J-point. Summations of the absolute values of the ST-segment shifts from baseline on surface and on intracoronary ECGs were calculated separately and as overall ST-segment changes (surface plus intracoronary ECG) and expressed in millimeters (1 mm=0.1 mV).

Assessment of Cardiac Pain

At the beginning of each coronary angioplasty procedure, patients were informed that they might develop chest pain. At the end of the first two balloon inflations, the intensity of cardiac pain was assessed by using a visual-analog scale. Patients were asked to put a mark on a 100-mm scale marked from no symptoms (0) to severe symptoms (100).

Determination of Blood Glucose Levels

Venous blood samples for the measurement of blood glucose levels, using routine glucose oxidase method, were obtained at baseline, 60 minutes after the administration of glibenclamide or placebo, and before the first balloon inflation.

Statistical Analysis

Two-factor ANOVA with repeated measures on one factor was used to compare ischemic ECG changes during balloon inflations in the two groups of patients. When significant differences were detected, pairwise comparisons were made using the Scheffé F test. Comparisons of the remaining continuous or discrete variables between the two groups were performed using an unpaired Student’s t or a χ² test, respectively. Visual-analog scales were analyzed using the Wilcoxon signed rank test or the Mann-Whitney U test as appropriate. Data are expressed as mean±1 SD; values of P<.05 were considered significant.

Results

Clinical, anatomic, ECG, and hemodynamic features in the two groups of patients are summarized and presented in the Table.

Coronary Angioplasty

Coronary angioplasty was successfully performed in all 20 patients. The mean balloon pressure was similar in glibenclamide- and placebo-treated patients (5±1 versus 5±2 atm, respectively; P=NS). Coronary stenosis was reduced from 90±11% to 22±11% (P<.001) in the glibenclamide-treated group and from 88±7% to 20±7% (P<.001) in the placebo-treated group. The recovery period between the two balloon inflations was similar for glibenclamide- and placebo-treated patients (8±2 versus 8±1 minutes, respectively; P=NS). All procedures were free of complications, with no ECG or enzymatic evidence of myocardial injury.

Myocardial Ischemia

In the glibenclamide-treated patients, the mean ST-segment shift during the second balloon inflation was similar to that during the first inflation on the surface ECG (11±8 versus 11±8 mm, P=NS), the intracoronary ECG (12±8 versus 8±6 mm, P=NS), and the surface plus intracoronary ECGs (23±13 versus 20±8 mm, P=NS). Conversely, in placebo-treated patients, the mean ST-segment shift during the second balloon inflation was significantly less than that during the first inflation on the surface ECG (5±3 versus 9±4 mm, P<.001), the intracoronary ECG (4±3 versus 11±6 mm, P<.001), and the surface plus intracoronary ECGs (9±5 versus 23±13 mm, P<.001) (Fig 1). Of note, there was no significant difference between the two groups of patients in the degree of ST-segment shift at the end of the first inflation on either surface (P=NS) or intracoronary ECG (P=NS) (Table).

Cardiac Pain

In glibenclamide-treated patients, the severity of cardiac pain during the second inflation was significantly greater than that during the first inflation (55±21 versus 43±23 mm, P<.05). Conversely, in placebo-treated pa-
Clinical, Anatomic, ECG, and Hemodynamic Features in the Two Groups of Patients

<table>
<thead>
<tr>
<th>Age, y</th>
<th>Glibenclamide (n=10)</th>
<th>Placebo (n=10)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>9/1</td>
<td>8/2</td>
<td>NS</td>
</tr>
<tr>
<td>Vessel disease, %</td>
<td>LAD</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>LCx</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>RCA</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Severity of stenosis, %</td>
<td>Before PTCA</td>
<td>90±11</td>
<td>88±7</td>
</tr>
<tr>
<td></td>
<td>After PTCA</td>
<td>22±11*</td>
<td>20±7*</td>
</tr>
<tr>
<td>Blood glucose levels, mg/100 mL</td>
<td>Baseline values</td>
<td>102±10</td>
<td>96±12</td>
</tr>
<tr>
<td></td>
<td>60 Min after treatment</td>
<td>53±9‡</td>
<td>96±10</td>
</tr>
<tr>
<td></td>
<td>Before PTCA</td>
<td>95±19</td>
<td>96±11</td>
</tr>
<tr>
<td>RPP, beats per minute per mm Hg×10³</td>
<td>Inflation 1</td>
<td>9±3</td>
<td>11±4</td>
</tr>
<tr>
<td></td>
<td>Inflation 2</td>
<td>9±3</td>
<td>11±3</td>
</tr>
<tr>
<td>ST-segment shift on S-ECG, mm</td>
<td>Inflation 1</td>
<td>11±8</td>
<td>9±4</td>
</tr>
<tr>
<td></td>
<td>Inflation 2</td>
<td>11±8</td>
<td>5±3‡</td>
</tr>
<tr>
<td>ST-segment shift on IC-ECG, mm</td>
<td>Inflation 1</td>
<td>8±6</td>
<td>11±6</td>
</tr>
<tr>
<td></td>
<td>Inflation 2</td>
<td>12±8</td>
<td>4±3‡</td>
</tr>
<tr>
<td>Pain severity, mm</td>
<td>Inflation 1</td>
<td>43±23</td>
<td>42±19</td>
</tr>
<tr>
<td></td>
<td>Inflation 2</td>
<td>55±21§</td>
<td>15±15</td>
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</tbody>
</table>

LAD indicates left anterior descending coronary artery; LCx, left circumflex coronary artery; RCA, right coronary artery; PTCA, percutaneous transluminal coronary angioplasty; RPP, rate-pressure product (heart rate×systolic arterial pressure); S-ECG, surface 12-lead ECG; and IC-ECG, intracoronary ECG.

*P<.001 vs stenosis before PTCA; †P<.001 vs baseline value; §P<.001 vs inflation 1 value; ‡P<.05 vs inflation 1 value; ‖P<.01 vs inflation 1 value.

Fig 1. Plots of individual values of ST-segment shifts on the surface ECG (S-ECG) and the intracoronary ECG (IC-ECG) at the end of the first and second balloon inflations in the two groups of patients. In glibenclamide-treated patients, ST-segment changes at the end of the second balloon inflation were similar to those at the end of the first inflation. Conversely, in placebo-treated patients, ST-segment changes at the end of the second balloon inflation were significantly less than those at the end of the first inflation.

**Discussion**

This study confirms that in humans, during coronary angioplasty, the severity of myocardial ischemia, as assessed using both intracoronary and standard 12-lead ECGs, during the second balloon inflation is less than that during the first inflation. Moreover, it shows that this effect is entirely prevented by pretreatment with glibenclamide, a selective K<sub>ATP</sub> channel blocker. Therefore, our findings indicate that in humans ischemic preconditioning during brief repeated coronary occlusions is mediated by the activation of K<sub>ATP</sub> channels, which is in agreement with the results of several experimental studies. In the present study, we also evaluated the impact of various conditions on cardiac pain severity.
of the $K_{ATP}$ channel blockade on cardiac pain, as assessed using a visual-analog scale, a well-accepted method for the evaluation of pain perception\textsuperscript{28} that we have used successfully in several previous studies.\textsuperscript{21,26-28} We found that in glibenclamide-treated patients the anginal pain during the second balloon inflation was even greater than that during the first inflation, thus confirming the trend toward a worsening of the signs of myocardial ischemia observed on the intracoronary ECG. Conversely, in placebo-treated patients, the anginal pain during the second balloon inflation was less severe, thus confirming that the lesser severity of ischemic ECG changes reflected a lesser degree of myocardial ischemia.

We cannot exclude that glibenclamide prevented the reduction of myocardial ischemia during the second balloon inflation independent of $K_{ATP}$ channel blockade in the myocardium. In fact, high doses of intracoronary glibenclamide have been demonstrated to decrease coronary blood flow and to interfere with hypoxic and ischemic vasodilation by blockade of $K_{ATP}$ channels in vascular smooth muscle.\textsuperscript{29,30} However, if glibenclamide had prevented ischemic vasodilation, we should also have obtained greater ECG changes and more severe pain in glibenclamide-treated patients at the end of the first inflation. Instead, the magnitude of ischemic ECG changes and the severity of pain at the end of the first inflation were similar in glibenclamide- and placebo-treated patients. Another theoretical possibility is that glibenclamide prevented collateral recruitment during the second inflation. However, glibenclamide has been shown to block ischemic preconditioning in dogs independent of effects on collateral flow\textsuperscript{10} and to block preconditioning in pigs, a species with essentially no resting collateral flow.\textsuperscript{16} Moreover, in anesthetized dogs, Auchampach et al\textsuperscript{11} demonstrated that there was no difference in the transmural collateral blood flow, to the ischaemic and reperfused left anterior descending coronary artery region among groups treated with aprikalim, a $K_{ATP}$ channel opener; glibenclamide; or placebo; this occurred despite improvement with aprikalim and worsening with glibenclamide of the contractile function after ischemia. Finally, Deutsch et al\textsuperscript{19} found that the reduction of ischemia during the second of two consecutive 90-second periods of coronary artery balloon occlusion in humans was associated with unchanged coronary wedge pressure. Therefore, in our study it also is very unlikely that glibenclamide prevented preconditioning by preventing collateral recruitment. Finally, glibenclamide might impair preconditioning by reducing blood glucose levels. However, this can be excluded in our study, as blood glucose levels were corrected and were similar before coronary angioplasty in the glibenclamide- and placebo-treated patients.

Experimental studies suggest that $K_{ATP}$ channel activation in myocardial fibers limits the detrimental effects of ischemia, probably because of the shortening of the action potential duration, which in turn results in a reduction in calcium inflow and ATP depletion.\textsuperscript{31-34} How the activation of $K_{ATP}$ channels during a brief ischemic period protects the myocardium during subsequent short or long periods of ischemia, occurring in the next few hours or even after 24 hours, remains unknown. Our results do not provide answers to these critical questions; they also do not explore the role played by adenosine A$_1$ receptors,\textsuperscript{35-38} G proteins,\textsuperscript{39-41} $\alpha$-adrenergic receptors,\textsuperscript{42} or muscarinic receptors\textsuperscript{43} in the complex phenomenon of preconditioning. Nevertheless, to the best of our knowledge, this study is the first to show that $K_{ATP}$ channels play a key role in preconditioning in man, at least in the model we used.

**Study Limitations**

A limitation of this study is the use of a single-blind design. However, the selection of objective ECG end points and the analysis of the results blind to treatment should substantially overcome the drawbacks of the single-blind design. The presence of concomitant pharmacological agents—aspirin, calcium channel blockers, and long-acting nitrates—is an additional confounding factor. However, all patients were taking the same pharmacological agents, and therefore it is unlikely that this may have influenced the results relative to the comparisons between glibenclamide- and placebo-treated patients. Another potential limitation of this study is the short balloon inflation duration of only 2 minutes, whereas in the majority of experimental studies longer ischemic periods have been used to produce preconditioning. However, both experimental\textsuperscript{42,44} and clinical\textsuperscript{19} studies have demonstrated that a coronary occlusion period of 2 minutes or 90 seconds, respectively, is sufficient to obtain ischemic preconditioning.

Finally, we based the assessment of myocardial ischemia only on the ECG changes and on the anginal pain severity. However, the surface 12-lead and the intracoronary ECGs represent high-sensitive, well-accepted, and simple methods for the evaluation of myocardial ischemia during coronary angioplasty.\textsuperscript{19,21,45-49} In particular, Labovitz et al\textsuperscript{48} found that during coronary angioplasty ST-segment changes always precede left ventricular systolic dysfunction. Moreover, we compared ST-segment changes in the same patient, where with other variables constant, the most important parameter determining the degree of ST-segment shift appears to be the severity of myocardial ischemia, as previously shown in experimental studies.\textsuperscript{50,51} In our study, a confounding factor in the interpretation of the ischemic ECG changes is the direct electrophysiological effect of glibenclamide. The opening of $K_{ATP}$ channels, however, leads to elevation of the ST segment, probably due to a shortening of the action potential duration preferentially in the epicardial layers, whereas this effect is prevented by glibenclamide.\textsuperscript{50,52-55} It follows that glibenclamide per se may lead, if anything, to an underestimation of the blockade of preconditioning observed in our patients. Such an underestimation may explain why in glibenclamide-treated patients the anginal pain was more severe during the second inflation, whereas the degree of ST-segment shift did not increase significantly.

**Clinical Implications**

The results of our study may have some important clinical implications. They suggest that the prevention of preconditioning may explain the mortality excess from cardiovascular causes in diabetic patients on sulfonylureas\textsuperscript{56} and the worse outcome of patients who are on sulfonylureas at the time of acute myocardial infarction.\textsuperscript{57,58} Previous studies\textsuperscript{57,58} and our findings appear, therefore, to suggest that the treatment of diabetes in
some high-risk coronary patients should be shifted from sulfonylureas to insulin, as recently proposed. However, because our study assessed the effects of a single dose of glibenclamide (similar to the maximal daily dosage used in diabetic patients), we cannot extrapolate our results to the effects of chronic treatment with sulfonylureas, as the responsiveness of $K_{ATP}$ channels during chronic treatment with glibenclamide may, in theory, change. Finally, this study suggests that the enhancement of preconditioning by $K_{ATP}$ channel openers might limit the detrimental effects of myocardial ischemia in humans.

Acknowledgments

We are deeply grateful to Prof Attilio Masi, FRCP, and Icilio Cavero, PhD, for their comments and constructive criticism. We wish also to thank Mrs Teresa Palumbo, RN, and Miss Emma Tomsett for invaluable technical assistance.

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Ischemic preconditioning during coronary angioplasty is prevented by glibenclamide, a selective ATP-sensitive K+ channel blocker.
F Tomai, F Crea, A Gaspardone, F Versaci, R De Paulis, A Penta de Peppo, L Chiariello and P A Gioffrè

_Circulation_. 1994;90:700-705
doi: 10.1161/01.CIR.90.2.700

_Circulation_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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