Effects of Tolbutamide on Vascular ATP-Sensitive Potassium Channels in Humans

Comparison With Literature Data on Glibenclamide and Glimepiride

P. J. Bijlstra¹, F. G. M. Russel², T. Thien¹, J. A. Lutterman¹ and P. Smits¹, ²

¹ Department of Internal Medicine and ² Department of Pharmacology, University Hospital Nijmegen, Nijmegen, The Netherlands

Sulfonylurea (SU) derivatives exert their hypoglycemic effect by blockade of adenosine-5'-triphosphate-sensitive potassium (K\textsubscript{ATP}) channels in the beta cell of the pancreas. Interestingly, K\textsubscript{ATP} channels also occur in the cardiovascular system, where they are thought to play an important role in cardioprotective mechanisms against ischemia. We have recently shown that the classical second generation SU-derivative glibenclamide is able to block vascular K\textsubscript{ATP} channels in man, whereas the newly developed second generation derivative glimepiride was devoid of this property. The aim of this study was to determine whether the first generation SU derivative tolbutamide has K\textsubscript{ATP} channel blocking properties in humans. In a group of 12 healthy male non-smoking volunteers, we investigated whether therapeutic concentrations of tolbutamide were able to inhibit the forearm vasodilation in response to the infusion of the K\textsubscript{ATP} channel opening drug diazoxide into the brachial artery. Changes in forearm blood flow were recorded by venous occlusion mercury-in-silastic strain-gauge plethysmography. Diazoxide alone increased the forearm blood flow ratio dose-dependently by ultimately 691 ± 198 %. A second diazoxide infusion in the presence of tolbutamide revealed a comparable vasodilator response with a percentage increase in forearm blood flow ratio of ultimately 542 ± 111 %. This response did not differ from the vasodilator response to diazoxide alone. The present study shows that therapeutic concentrations of tolbutamide are not able to attenuate the vasodilation caused by the K\textsubscript{ATP} channel opener diazoxide in man. When compared with published data on second generation SU-derivatives, tolbutamide shows an intermediate position between glibenclamide (with significant blockade of vascular K\textsubscript{ATP} channels) versus glimepiride (with no blockade at all). It remains to be determined whether these acute effects of SU derivatives on pharmacological opening of forearm vascular K\textsubscript{ATP} channels can be extrapolated to the chronic effects of these drugs on ischemia-mediated opening of myocardial K\textsubscript{ATP} channels during treatment of NIDDM patients.

Key words: Tolbutamide – Glibenclamide – Glimepiride – Diazoxide – Forearm Blood Flow – Cardioprotection – Sulfonylurea Derivatives – Potassium Channels – Vascular

Abbreviations

K\textsubscript{ATP}Channel = adenosine-5'-triphosphate-sensitive potassium channel
SU = sulfonylurea
FBF = forearm blood flow
FVR = forearm vascular resistance
MAP = mean arterial pressure

Introduction

Since the introduction of sulfonylurea (SU) derivatives, studies of these agents have been focused on metabolic effects in patients with non-insulin dependent diabetes mellitus (NIDDM) (11,17). Both the first generation SU derivative tolbutamide and the second generation SU derivative glibenclamide induce their pharmacological effect by blockade of adenosine-5'-triphosphate-sensitive potassium (K\textsubscript{ATP}) channels in the beta cell of the pancreas. This ultimately promotes an influx of calcium with subsequent stimulation of insulin release (9). In the last two decades, an increasing interest in the extra-pancreatic effects of these drugs has been reported (1,3,19,21,22,24). In animal models, SU derivatives have been shown to attenuate the vasodilator response to an ischemic stimulus (2), and to abolish the protective effects of myocardial ischemic preconditioning (12,26). In these preparations, glibenclamide has even been shown to increase myocardial infarct size (25). In theory, the aforementioned observations may suggest harmful cardiovascular effects of SU derivatives when used under conditions of ischemia in patients with NIDDM. In 1970 the University Group Diabetes Program (UGDP) study first reported possible adverse cardiovascular effects of SU derivatives in man (27). Although this study has been extensively criticized, recent observations on the interaction between SU-derivatives and human vascular K\textsubscript{ATP}-channels may be of great importance with respect to this issue (5).

We have just shown that the classical second generation SU derivative glibenclamide can interact with vascular K\textsubscript{ATP} channels in man at therapeutic concentrations (5). In theory, this interaction may have contributed to the cardiovascular adverse effects as reported in the UGDP-study (5). However, it has to be emphasized that tolbutamide and not glibenclamide was used in the UGDP-study. Almost all experimental animal studies on cardiovascular effects of SU-derivatives have used glibenclamide, and not tolbutamide. Therefore, we conducted...
a study to evaluate whether therapeutic concentrations of tolbutamide are able to block vascular K_ATP-channels in humans. This question was investigated by recording the interaction of tolbutamide with the K_ATP-channel opening drug diazoxide. The data will be discussed in relation to previously published original observations on glibenclamide (5). Data on the newly developed second generation SU-derivative glimepiride will also be discussed, since this agent seems to be devoid of K_ATP-channel blocking properties (5,14,15).

Methods

This study investigated the putative inhibitory effects of tolbutamide on the K_ATP-channel-mediated vasodilation by diazoxide. To address this issue, the perfused forearm technique was used (28). The study protocol was approved by the local ethics committee, and all 12 participants gave written informed consent before entering the study. All experiments were performed in healthy male non-smoking volunteers with a normal history, physical examination, and blood pressure. The characteristics of the volunteers are listed in Table 1. Each volunteer participated in only one experiment and was instructed to abstain from caffeine-containing beverages and alcohol at least 24 hours before the experiment. Furthermore, they were asked to eat a light meal two hours before the experiment was started and then to abstain from further food intake until after the experiment. Forearm volume was measured by water displacement. The experiments were performed with the subjects in the supine position in a quiet temperature controlled room (22°C), to ensure that forearm blood flow (FBF) predominantly reflected forearm muscle perfusion (8).

Table 1  Characteristics of the healthy volunteers.

<table>
<thead>
<tr>
<th>n</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>26 ± 7</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>76 ± 13</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.84 ± 0.06</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>22 ± 3</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)*</td>
<td>123 ± 6</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)*</td>
<td>65 ± 7</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>62 ± 6</td>
</tr>
</tbody>
</table>

Data are presented as means (± SD).

*Phygmomanometrically obtained blood pressure after 5 min supine rest.

The effects of tolbutamide on the diazoxide-mediated vasodilation were studied. A cannula was inserted into the brachial artery (Angiocath, 20 gauge, Deseret Medical Inc., Becton Dickinson and Comp., Sandy, Utah). Antecubital veins of the left and the right arm were also cannulated. After an equilibration period of 45 minutes, a 35 minute intraarterial infusion of placebo (saline) was started. After 10 minutes of saline infusion, baseline measurements of blood pressure, heart rate, and bilateral FBF were taken during the concomitant intraarterial infusion of placebo. Then a dose-response curve of the vasodilator effects of diazoxide was made (three intraarterial dosages: 0.25, 0.75, 2.25 mg/min/dl using an automated syringe infusion pump) (type STC-521, Terumo Corp., Tokyo, Japan). Blood pressure was measured via the intraarterial line using a Hewlett Packard monitor (type 7853B, Hewlett Packard GmbH, Böblingen, Germany). Forearm blood flow was measured by ECG-triggered venous occlusion mercury-in-silastic strain-gauge plethysmography (Hokanson EC4, D.E. Hokanson, Inc., Issaquah, Washington, USA). During all recordings of the FBF, the hand circulation was completely occluded by a wrist cuff inflated 100 mmHg above the systolic blood pressure to be sure that measurements only referred to the forearm skeletal muscle vascular bed (16). After a subsequent equilibration of 60 minutes to allow parameters to return towards baseline levels, baseline values were again recorded during concomitant intraarterial infusion of placebo. Then a second dose-response curve was made in all subjects, but now with concomitant intraarterial infusion of tolbutamide instead of placebo (tolbutamide dose 1.0 mg/min/dl).

Venous blood samples from the non-experimental arm were taken for the determination of insulin and C-peptide as well as for tolbutamide concentrations, both before and at the end of tolbutamide infusion. After each diazoxide infusion, venous blood samples from the experimental side were taken for the determination of local tolbutamide concentrations. At all these time points, the plasma glucose concentration was also measured. Glucose was measured immediately by an Accutrend glucose analyzer (type 1284851, Boehringer, Mannheim, Germany). Because of the long half-life, high protein binding (7,23) and possible incorporation of SU derivatives in the plasma membrane (20), randomization of placebo and tolbutamide was not possible because of carry-over effects.

For comparison, we also discuss here the data from identical studies with glibenclamide, which was given in a dose of 0.33 μg/min/dl, and from studies with the newly developed SU derivative glimepiride, administered in a relatively high dose of 2.5 μg/min/dl (5).

Humoral parameters

For determinations of local serum concentrations of the SU derivative, venous blood samples were collected in glass tubes without additives. After 20 minutes the blood was centrifuged at 3000 rpm for 10 minutes. Then serum was frozen at -20°C. The drug concentrations in these serum samples were determined at the laboratories of Hoechst Marion Roussel, Frankfurt, Germany.

Insulin and C-peptide were measured in venous blood samples collected in chilled glass tubes coated with lithium-heparin. The blood was centrifuged at 3000 rpm for 10 minutes. Then plasma was frozen at -20°C. In these samples, insulin and C-peptide were determined in our laboratories using specific RIAs. C-Peptide was measured with a standard kit (D.P.C., Los Angeles, CA, USA) and insulin with a procedure using standard and tracer prepared from monocomponent human insulin (NOVO, Zoeterwoude, The Netherlands).

Calculations and statistics

Forearm vascular resistance (FVR) was calculated as the quotient of mean arterial pressure (MAP) and FBF. The ratio of experimental to non-experimental FBF was calculated to correct for systemic changes due to time or arousal in order to refer only to changes induced by local infusions (10). For each dose of diazoxide, FBF values in the last two minutes of infusion were averaged to one mean representative value. Likewise, absolute and percentage changes from baseline were calculated.
Table 2 Mean (±SE) baseline values of forearm blood flow and vascular resistance, and the absolute and percentage changes of these parameters induced by diazoxide infusion, both during concomitant placebo (upper part) or tolbutamide infusion (lower part). AU: Arbitrary Units

Table 3 Mean (±SE) plasma concentrations of insulin, C-peptide and glucose at different time points throughout the study period.

In the same manner, values for FVR and FBF-ratio were obtained. The results were analyzed statistically by an analysis of variance (ANOVA) with repeated measures over all vasodilator dosages. A p-value of <0.05 was considered statistically significant. Because of significant differences in baseline values, this analysis was performed on the percentage changes from baseline for the FBF-ratio, but also for the FBF and FVR, followed by post-hoc t-tests when ANOVA was significant. Values presented are means ±SE unless indicated otherwise.

**Results**

**Effects of sulfonylurea derivatives on vasodilator responses**

During concomitant placebo infusion, the three dosages of diazoxide increased the FBF at the experimental arm dose-dependently by 99±24, 178±34 and 457±119% respectively. Changes at the contralateral arm were negligible: -4±4, -13±5 and -23±5% respectively. After equilibration, FBF returned to baseline levels, and the subsequent infusion in the presence of tolbutamide increased FBF by 61±14, 146±33 and 451±80% respectively. This second dose response curve was not different from the first one. Table 2 shows the absolute changes in FBF as well as in FVR for all dose steps. Fig. 1 illustrates the changes in FBF-ratio induced by diazoxide infusion into the brachial artery, both in the presence of placebo or tolbutamide. The right panel of this figure shows the percentage changes from baseline in FBF-ratio. The p-value refers to ANOVA with repeated measures over all diazoxide dosages.

**Changes in humoral parameters**

Table 3 shows the concentrations of glucose, insulin and C-peptide throughout the study. As a result of the regional infusion and the relatively low cumulative dose of tolbutamide, no increase in systemic insulin or C-peptide concentrations and no fall in plasma glucose levels were observed during this study.

During the second dose response curve, tolbutamide concentrations were sampled at the end of each diazoxide dose. These local serum tolbutamide concentrations averaged 26±4 μg/ml, 17±2 μg/ml and 11±1 μg/ml respectively (n = 12). At the end of the experiment the systemic tolbutamide concentration had accumulated to 5±0 μg/ml.
The present study shows that the first generation SU derivative tolbutamide does not significantly attenuate the vasodilator response to the K_{ATP} channel opener diazoxide in man. The intraarterial dosages of tolbutamide resulted in local concentrations which were well within the therapeutic range as they occur systemically during regular treatment of NIDDM (18). These observations argue against a significant effect of tolbutamide treatment on vascular K_{ATP} channels in humans.

In theory, the present observations may have been confounded by time effects. However, in our previous study on this issue we carefully performed time control studies, which showed that the repeat of diazoxide infusion in the time frame used in the current study resulted in an identical forearm vasodilator response (5).

When the effect of tolbutamide on the three vasodilator dosages to diazoxide is averaged to one mean value, a simple comparison can be made with the previously published data on glibenclamide and glimepiride (5). For the sake of comparison, data are depicted in Fig. 2. All these experiments have been performed in exactly the same way, and they all refer to groups of 12 volunteers. From this figure it is clear that tolbutamide has an intermediate position between glibenclamide and glimepiride. For glibenclamide the blockade of vascular K_{ATP} channels was statistically significant (5), whereas glimepiride showed no blockade at all.

The relative absence of an interaction with vascular K_{ATP} channels by tolbutamide can easily be explained by its weak pharmacological effect, which also holds for the insulinotropic effect of this drug. As soon as metabolic regulation of NIDDM is insufficient with tolbutamide, the switch to a second generation SU-derivative is indicated. In this regard it is interesting that a switch to glimepiride will clearly improve metabolic regulation, whereas the function of the vascular K_{ATP} channels will not be impaired by this agent.

Patients with NIDDM are relatively often exposed to myocardial ischemia. Blockade of vascular K_{ATP} channels by glibenclamide has been shown to impair K_{ATP} channel-mediated protective effects (6,12). The UGDP-study suggested that tolbutamide may have adverse cardiovascular effects (27). This has also been suggested for glibenclamide (13,24). Our present study suggests that after acute administration, tolbutamide will probably not attenuate K_{ATP} channel-mediated cardioprotective mechanisms. However, we can not exclude the possibility that chronic exposure to tolbutamide, such as occurs during the treatment of NIDDM, may result in significant blockade of vascular K_{ATP} channels in man. The present study must be interpreted as a call for further studies on the effects of chronic treatment with SU-derivatives on K_{ATP} channel-mediated effects in patients with NIDDM.

Conclusions

We conclude that therapeutic concentrations of the first generation SU derivative tolbutamide are not able to attenuate vascular K_{ATP} channels in humans.
Requests for reprints should be addressed to:
Paul Smits, MD, PhD
Professor of Clinical Pharmacology
Department of Pharmacology
University of Nijmegen
P.O. Box 9101
NL-6500 HB Nijmegen
The Netherlands