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Effects of fluoroquinolones on insulin secretion and β-cell ATP-sensitive K^+ channels

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Abstract

Although fluoroquinolones are used widely in the treatment of various infectious diseases, some of the drugs are known to cause hypoglycemia as a side-effect. We have investigated the effects of three fluoroquinolone derivatives, levofloxacin, gatifloxacin, and temafloxacin, on insulin secretion and pancreatic β-cell ATP-sensitive K^+ channel (K_{ATP} channel) activity. While levofloxacin had only a small effect on insulin secretion and K_{ATP} currents, gatifloxacin and temafloxacin stimulated insulin secretion and inhibited K_{ATP} channel currents in a dose-dependent manner. We also determined the site of action of gatifloxacin and temafloxacin on the K_{ATP} channel. In a reconstituted system, gatifloxacin and temafloxacin inhibited Kir6.2ΔC26 channels, which function in the absence of the SUR subunit, indicating direct action of the drugs on the Kir6.2 subunits. These results suggest that stimulation of insulin secretion by inhibition of pancreatic β-cell K_{ATP} channels underlies the hypoglycemia caused by certain fluoroquinolones.

Keywords: ATP-sensitive K^+ channel; Insulin secretion; Fluoroquinolone; Levofloxacin; Gatifloxacin; Temafloxacin

1. Introduction

The fluoroquinolone class of antibacterial agents is used widely in the treatment of various infectious diseases because of their broad spectrum and strong antibacterial activities. While this class of antibacterial agent is generally well tolerated and effective, the drugs have side-effects including central nervous system (CNS) toxicity, phototoxicity, and QT prolongation (Domagala, 1994; Ball et al., 1999). Some fluoroquinolones, e.g., lomefloxacin and gatifloxacin, have been reported to induce hypoglycemia (Rubinstein, 2001; Baker and Hangii, 2002). In vitro studies found that lomefloxacin stimulates insulin secretion from rat pancreatic islets (Maeda et al., 1996), and that the drug inhibits K_{ATP} channel activity in the clonal insulinoma cell line RINm5F (Zünkler and Wos, 2003). In addition, quinine and mefloquinine, both of which are structurally similar to quinolone, an anti-malarial agent, have been shown to cause hypoglycemia by stimulating insulin secretion by inhibiting K_{ATP} channel activity (Henquin, 1982; Bokvist et al., 1990; Gribble et al., 2000). The molecular mechanism of fluoroquinolone-induced hypoglycemia is not known, however.

K_{ATP} channels in pancreatic β-cells are crucial in the regulation of insulin secretion. Closure of the channels depolarizes the β-cell membrane, opening the voltage-dependent calcium channels to allow calcium influx. The resultant intracellular Ca^{2+} increase triggers exocytosis of insulin granules (Ashcroft and Rorsman, 1989). The sulphonylureas used in treatment of type 2 diabetes mellitus (Sturgess et al., 1985; Ashcroft and Gribble, 1999), the imidazoline drugs such as phentolamine, used for peripheral vasodilation (Plant and Henquin, 1990; Proks and Ashcroft,

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1997), and cibenzoline, used as an anti-arrhythmic drug (Mukai et al., 1998), also inhibit the K_{ATP} channels in pancreatic β-cells. The pancreatic β-cell K_{ATP} channel is an octameric protein composed of SUR1 and Kir6.2 subunits (Inagaki et al., 1995; Aguilar-Bryan et al., 1995), and drugs that inhibit K_{ATP} channels act on the SURx regulatory subunits (SUR1 or SUR2) or the Kir6.x pore-forming subunits (Kir6.2 or Kir6.1).

In the present study, we demonstrate differing effects of fluoroquinolones on insulin secretion and pancreatic β-cell K_{ATP} channels. Gatifloxacin and temafloxacin inhibit pancreatic β-cell K_{ATP} channels and stimulate insulin secretion significantly, while levofloxacin has little effect. These findings also clarify the mechanism by which fluoroquinolones induce hypoglycemia.

2. Materials and methods

2.1. Materials

Levofloxacin, gatifloxacin, and temafloxacin (Fig. 1) were synthesized at Daiichi Pharmaceutical and dissolved in 0.1 N NaOH or KOH to obtain stock solution of 30 mM.

2.2. Isolation of mouse pancreatic islets and batch incubation experiments

All animal procedures were approved by Chiba University Animal Care Committees. Mouse pancreatic islets were isolated by a collagenase digestion method described previously (Miki et al., 1998), and cultured in RPMI-1640 medium (glucose concentration, 11.1 mM) containing 10% fetal bovine serum under a humidified condition of 5% CO_2–95% air at 37 °C for 24 h. Batch incubation experiments were performed as described previously (Miki et al., 1998; Kashima et al., 2001). Briefly, after preincubation (30 min) with HEPES–Krebs Buffer containing 2.8 mM glucose, five size-matched islets were collected in each tube and incubated in 500 μl of the same buffer containing 5.5 or 11.1 mM glucose with or without test compounds at the indicated concentrations for 30 min. Insulin released into the medium was measured by radio-immunoassay (Eiken Chemical, Tokyo, Japan).

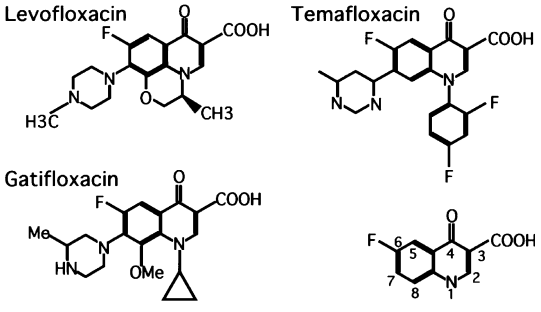

Fig. 1. Chemical structures of levofloxacin, gatifloxacin, and temafloxacin. The numbering convention of the fluoroquinolone structure is also shown.

2.3. Cell culture and transfection

MIN6m9 cells (Minami et al., 2000) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 25 mM glucose, 50 μM 2-mercaptoethanol, and 10% fetal bovine serum under a humidified condition of 5% CO_2–95% air at 37 °C. For single channel analysis, pCMVhKir6.2 (human Kir6.2 in a mammalian expression plasmid vector, pCMV6b) plus pCMVhSUR1 (human SUR1 in a pCMV6b), pCMVKir6.2ΔC26 (Tucker et al., 1997), and pCMVKir6.2ΔC26 plus pCMVhSUR1, respectively, were transfected into HEK-293T cells with Lipofectamine, Lipofectamine Plus Reagent and OPTI-MEM I reagents (Invitrogen, California), according to the instructions of the manufacturer, together with pEGFP-N1 as a reporter gene.

2.4. Electrophysiology

Whole-cell recordings of K_{ATP} currents were performed as described previously (Kawaki et al., 1999). The bath solution contained 135 mM NaCl, 5 mM KCl, 5 mM $CaCl_2$, 2 mM $MgSO_4$, 5 mM HEPES, and 2.8 mM glucose (pH 7.4). The pipette solution contained 107 mM KCl, 11 mM EGTA, 2 mM $MgSO_4$, 1 mM $CaCl_2$, 11 mM HEPES, and 0.3 mM ATP (pH 7.2, adjusted with KOH).

Single channel recordings of K_{ATP} currents were performed as described previously (Inagaki et al., 1995). The bath solution contained 140 mM KCl, 2 mM $MgCl_2$, 1 mM EGTA, 10 mM HEPES, and 1 μM ATP (pH 7.3). The pipette solution contained 140 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM HEPES (pH 7.3). Channel activity was calculated by integrating current flow during the channel openings for 5 s. Electrophysiological experiments were carried out at room temperature. We obtained the half-maximal concentration for inhibition (IC_{50}) and the Hill's coefficient (n_H) for gatifloxacin and temafloxacin by fitting the dose-inhibition data with the equation: channel activity (%)=100/(1+([fluoroquinolone]/IC_{50})^{nH}.

3. Results

3.1. Effects of various fluoroquinolones on insulin secretion

In the presence of 5.5 mM glucose, mouse pancreatic islets were incubated with levofloxacin, gatifloxacin, or temafloxacin. Levofloxacin had no significant effect on insulin secretion even at 300 μM. Gatifloxacin and temafloxacin at concentrations of 300 μM significantly increased insulin secretion. Similarly, in the presence of 11.1 mM glucose, gatifloxacin and temafloxacin increased

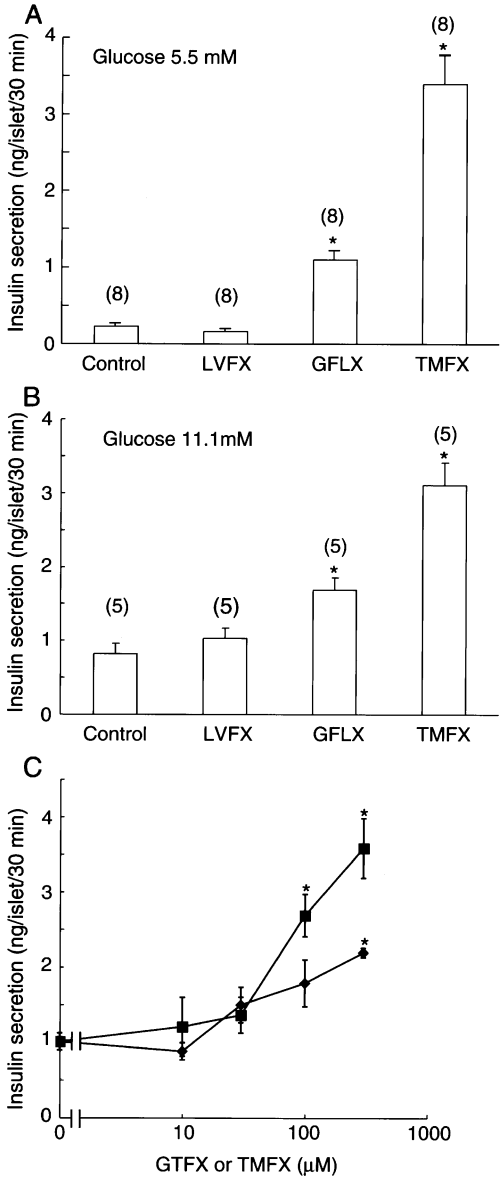

Fig. 2. Effects of levofloxacin, gatifloxacin, or temafloxacin on insulin secretion from mouse pancreatic islets. Islets were incubated for 30 min at 37 °C in medium containing 5.5 mM (A) or 11.1 mM (B) glucose with or without drugs. Control, no added drugs. LVFX, 300 μM levofloxacin. GFLX, 300 μM gatifloxacin. TMFX, 300 μM temafloxacin. Values are mean±S.E. Numbers in parentheses indicate *n* values. **P*<0.01 vs. control. Similar results were obtained in three independent experiments. (C) Insulin response to various concentrations of gatifloxacin (diamonds) or temafloxacin (squares) in the presence of 5.5 mM glucose. Values are mean±S.E. (*n*=4–5 at each concentration). **P*<0.01 vs. the values at 5.5 mM glucose alone. Similar results were obtained in two independent experiments.

insulin secretion, while levofloxacin had no significant effect on insulin secretion (Fig. 2B).

The addition of gatifloxacin or temafloxacin augmented the insulin release in a dose-dependent manner (Fig. 2C). Augmentation was significant ($P<0.01$) at concentrations greater than 100 and 300 μM, respectively.

3.2. Effects of various fluoroquinolones on K_{ATP} channel activity

To determine if the stimulatory effect on insulin secretion of fluoroquinolones is mediated by inhibition of K_{ATP} channel activity, whole-cell currents were recorded in insulin secreting β-cell line MIN6m9 cells (Minami et al., 2000). The MIN6m9 cell line is one of the few β-cell lines that retains insulin secretory response to glucose and other secretagogues, and has been used extensively in studies of the mechanisms of insulin secretion. A progressive increase in channel currents was detected in response to ±10 mV amplitude pulses from holding potential (−70 mV) when intracellular ATP was dialyzed by pipette solution. The increase in currents was reversibly inhibited by 100 μM tolbutamide (Fig. 3A). At a concentration of 300 μM, levofloxacin only slightly reduced K^+ channel currents,

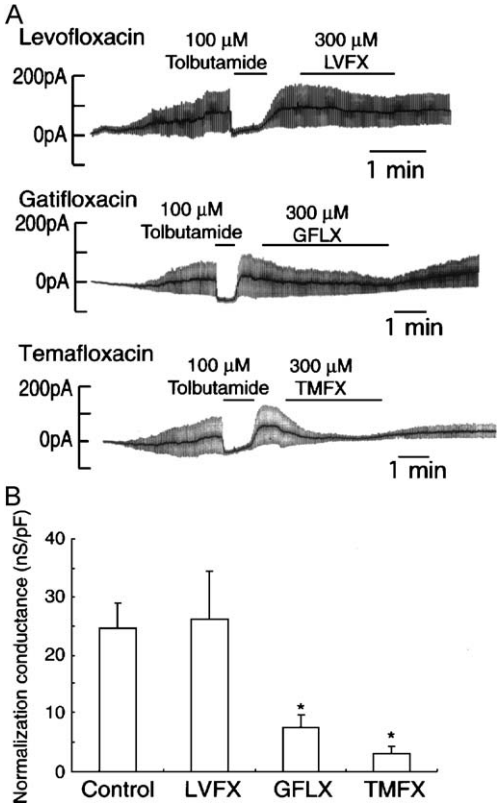

Fig. 3. Effects of levofloxacin, gatifloxacin, or temafloxacin on K_{ATP} channel currents from MIN6m9 cells. (A) Representative traces of whole-cell recordings of MIN6m9 cells. The holding potential was −70 mV and alternate voltage pulses of ±10 mV and 200 ms duration were applied every 2 s. Dialysis of the cells intracellularly with the pipette solution containing a low concentration of ATP (0.3 mM) caused a progressive increase in current. Horizontal bars indicate application periods of tolbutamide (100 μM), levofloxacin (300 μM), gatifloxacin (300 μM), and temafloxacin (300 μM), respectively. (B) Peak conductance of MIN6m9 cells measured in bath solution alone (control) and solution containing 300 μM levofloxacin (LVFX), 300 μM gatifloxacin (GFLX), or 300 μM temafloxacin (TMFX). Because the membrane area of each cell varies, conductance was normalized by dividing by the membrane capacitance measured for each cell. Values are mean±S.E. (*n*=4–15). **P*<0.01 vs. control.

while gatifloxacin or temafloxacin markedly inhibited K^+ currents (Fig. 3B). Blockade of K^+ currents with gatifloxacin or temafloxacin reversed slowly after washout of the drug.

The β-cell K_{ATP} channel is a heterooctamer composed four Kir6.2 subunits and four SUR1 subunits (Seino, 1999; Ashcroft and Gribble, 1998). To examine the effect of the drugs on β-cell K_{ATP} channel currents at the single-channel current level, we expressed Kir6.2 plus SUR1 channels in HEK-293T cells and recorded channel activity from membrane patches in the excised inside-out patch clamp mode. One mM ATP completely inhibited K_{ATP} channel current (Fig. 4Aa–c). Application of 300 μM levofloxacin to the bath solution had only a small effect on K_{ATP} channel current (Fig. 4Aa). Application of gatifloxacin or temafloxacin to the bath solution reduced K_{ATP} channel current in a dose-dependent manner (Fig. 4Ab,c). The relationship between the gatifloxacin or temafloxacin concentration and normalized K_{ATP} channel activity is shown in Fig. 4B. The data points were fitted by the Hill's equations. The half-maximal concentrations of the inhibition (IC_{50}) were 42.4 and 21.3 μM for gatifloxacin and temafloxacin, respectively. n_H values were 1.43 and 1.56, respectively.

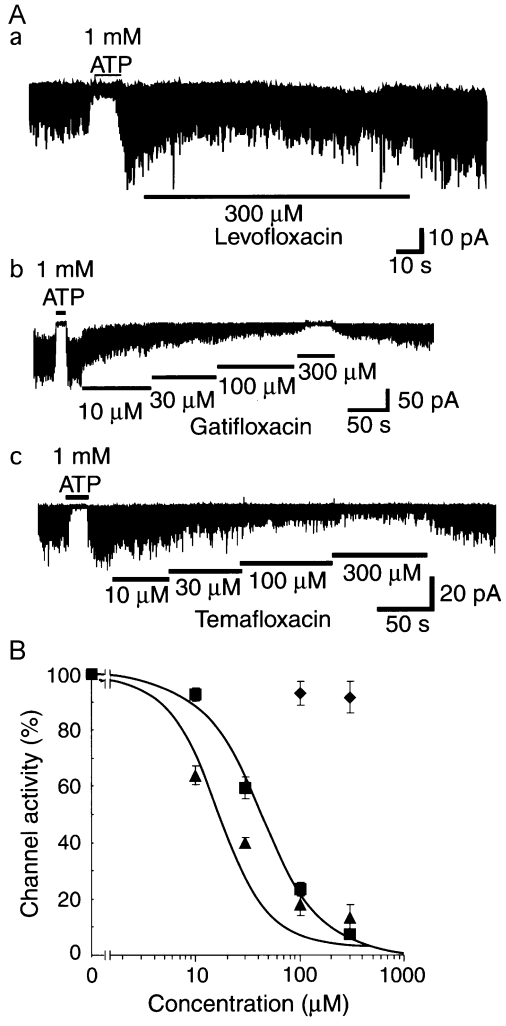

Fig. 4. Effects of levofloxacin, gatifloxacin, or temafloxacin on reconstituted K_{ATP} channel currents. (A) Representative current traces were recorded from HEK-293T cells coexpressing human Kir6.2 plus SUR1. Horizontal bars and numbers indicate application periods and concentration of ATP (panels a, b, and c), levofloxacin (a), gatifloxacin (b), and temafloxacin (c). (B) Dose-dependent inhibition of levofloxacin (diamonds), gatifloxacin (squares) and temafloxacin (triangles) measured in Kir6.2/SUR1 channels. Channel activity is expressed as percent of values in the presence of 1 μM ATP. The lines are drawn by fitting the Hill's equation. Values are mean±S.E. (n=5–6). Recordings were made in the inside-out patch configuration at membrane potential of −60 mV. ATP (1 μM) was always added in the bath solution.

We then determined whether gatifloxacin and temafloxacin inhibit K_{ATP} channel current by interacting with the Kir6.2 or SUR1 subunits of the K_{ATP} channels. Although Kir6.2 does not express functional K_{ATP} current when expressed alone, a mutant form of Kir6.2 with a C-terminal truncation of 26 amino acids (Kir6.2ΔC26) produces currents in the absence of SUR1 subunits (Tucker et al., 1997). Kir6.2ΔC26 thus provides a useful tool to identify drugs that act directly on the pore-forming subunit of the K_{ATP} channel. We expressed Kir6.2ΔC26 alone or Kir6.2ΔC26 plus SUR1 in HEK-293T cells and recorded channel activity in the excised patch membrane (Fig. 5).

K_{ATP} currents in HEK-293T cells expressing Kir6.2ΔC26 alone (Fig. 5Aa) were inhibited by application of gatifloxacin in a dose-dependent manner, similarly to those in Kir6.2ΔC26 plus SUR1 cells (Fig. 5Ac). The data points were well fitted with Hill's equations with IC_{50} values of 53.5 μM (n_H=1.11) for Kir6.2ΔC26 alone and IC50 values of 48.7 μM (n_H=1.31) for Kir6.2ΔC26 plus SUR1 (Fig. 5Ba). Similar results were obtained by application of temafloxacin (IC_{50} values of 41.1 μM and, n_H=1.06 for Kir6.2ΔC26 alone, and 38.94 μM and n_H=1.22 for Kir6.2ΔC26 plus SUR1) (Fig. 5Ab,d and Bb). These results indicate that both gatifloxacin and temafloxacin inhibit K_{ATP} channel activity by acting on the pore-forming Kir6.2 subunits.

4. Discussion

Fluoroquinolones are widely used as antibacterial drugs in the treatment of various infectious diseases. While many fluoroquinolone derivatives have proved useful as antibacterial agents, some of these drugs have been associated with serious side effects including phototoxicity, tendonitis, and QT interval prolongation (Rubinstein, 2001). In addition, some fluoroquinolones have been reported to induce hypoglycemia (Rubinstein, 2001; Menzies et al., 2002; Biggs, 2003) and to augment insulin release from rat pancreatic islets (Maeda et al., 1996). In the present study, we compared the effects of levofloxacin, gatifloxacin, and temafloxacin on insulin secretion and pancreatic β-cell K_{ATP} channel function. Levofloxacin (at concentrations up to 300 μM) had little effect on insulin secretion, while gatifloxacin and temafloxacin stimulated insulin secretion markedly. In

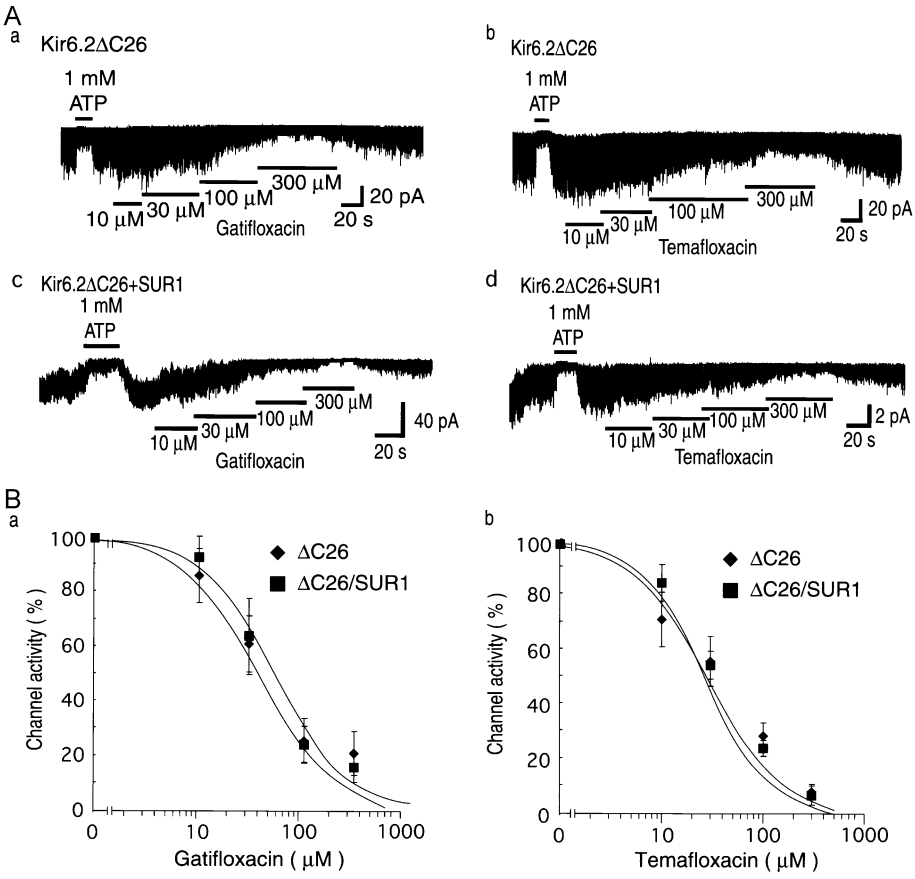

Fig. 5. Effects of gatifloxacin or temafloxacin on channel currents of Kir6.2ΔC26 and Kir6.2ΔC26 plus SUR1 channel activity. (A) Representative current traces recorded from HEK-293T cells expressing Kir6.2ΔC26 alone (a, b) or coexpressing Kir6.2ΔC26 plus SUR1 (c, d). Horizontal bars and numbers indicate application periods and concentration of ATP, gatifloxacin (a, c), and temafloxacin (b, d). (B) Dose-dependent inhibition of gatifloxacin (a) and temafloxacin (b) measured in Kir6.2ΔC26 (diamonds) and Kir6.2ΔC26/SUR1 (squares) channel activity. Channel activity is expressed as percent of control values before drug application. The lines are fitted by the Hill's equation. Values are mean±S.E. (n=4–5). The recordings were performed as in Fig. 4.

addition, gatifloxacin and temafloxacin inhibited both native K_{ATP} channels in β-cell line MIN6m9 cells and reconstituted K_{ATP} channels composed of Kir6.2 and SUR1 subunits. Gatifloxacin at a concentration of 300 μM almost completely inhibited K_{ATP} channel currents reconstituted in HEK-293T cells, as assessed by single channel recordings, while approximately 30% of the channel activity remained in MIN6m9 cells at the same concentration, as assessed by whole-cell recordings. The discrepancy could be due to partly to the different experimental conditions of reconstituted channels and native channels, including configuration of cell membrane. Our data demonstrate that while levofloxacin does not stimulate insulin secretion or affect channel function, gatifloxacin and temafloxacin stimulate insulin secretion by inhibiting pancreatic β-cell K_{ATP} channel activity directly.

Gatifloxacin and temafloxacin also inhibit the activity of K_{ATP} channels comprising Kir6.2ΔC26 subunits, which lack the C-terminal of Kir6.2 but nevertheless function as channels in the absence of SUR1 (Tucker et al., 1997). Both gatifloxacin and temafloxacin inhibited Kir6.2ΔC26 and Kir6.2ΔC26 plus SUR1 channels with similar IC_{50} and n_H values, indicating that the molecular target of gatifloxacin and temafloxacin is the Kir6.2 subunit rather than the SUR1 subunit. Kir6.2 is the pore-forming subunit of the K_{ATP} channel in β-cells, heart, skeletal muscle, and brain (Inagaki et al., 1995), where the K_{ATP} channels function as critical metabolic sensors under acute metabolic stress such as hyperglycemia, hypoglycemia, ischemia, and hypoxia (Miki et al., 2001; Suzuki et al., 2002; Yamada et al., 2001).

Administration of fluoroquinolone has been reported to be associated with prolongation of the QT interval on electrocardiogram (Jaillon et al., 1996; Morganroth et al., 1999). A possible mechanism of QT prolongation is blockade of human-ether-a-go-go related gene (HERG) channels or KvLQT/minK channels in heart (Curran et al., 1995; Sanguinetti et al., 1996). Fluoroquinolones have been shown to inhibit HERG channels in a wide range of potencies, such as an approximately 100-fold difference between sparfloxacin and ofloxacin (Kang et al., 2001). Application of lomefloxacin inhibits K_{ATP} channel currents in RINm5F cells, while application of norfloxain has only minor effects on K_{ATP} channel currents (Zünkler and Wos, 2003). The rank order of potency was found to be temafloxacin>gatifloxacin ⩾levofloxacin in the present study. These results demonstrate that fluoroquinolones differ

markedly in their ability to inhibit K^+ channel activity and to elicit insulin secretion. Of the three compounds examined, levofloxacin appears to be least likely to cause hypoglycemia when used clinically in the treatment of infectious diseases. In addition, because gatifloxacin and temafloxacin act directly on the Kir6.2 subunits, these drugs might affect heart, skeletal muscle, and hypothalamus function under certain conditions (Suzuki et al., 2001; Miki et al., 2001, 2002). Quinolinic acid is the basic structure of fluoroquinolones. The majority of the compounds have an oxygen attached at position 4 and a fluorine at position 6 (see Fig. 1). All of the other positions are capable of having different constituents. Each position around quinolinic acids determines specific activities such as pharmacodynamic effects and DNA gyrase, but also is responsible in the side-effect profiles (Domagala, 1994). Further studies are required to determine the molecular moieties involved in the inhibitory effects of fluoroquinolones on K_{ATP} channels.

In conclusion, we show here that while levofloxacin has only a minor effect on insulin secretion and K_{ATP} channel activity, gatifloxacin and temafloxacin both inhibit K_{ATP} channel activity directly by interacting with the Kir6.2 subunits of the channel, resulting in the stimulation of insulin secretion. These data indicate that fluoroquinolones should be used cautiously in the treatment of infections diseases.

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