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Effect of fluoroquinolones on mitochondrial function in pancreatic beta cells

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ABSTRACT

Hyper- and hypoglycaemias are known side effects of fluoroquinolone antibiotics, resulting in a number of fatalities. Fluoroquinolone-induced hypoglycaemias are due to stimulated insulin release by the inhibition of the K_{ATP} channel activity of the beta cell. Recently, it was found that fluoroquinolones were much less effective on metabolically intact beta cells than on open cell preparations. Thus the intracellular effects of gatifloxacin, moxifloxacin and ciprofloxacin were investigated by measuring NAD(P)H- and FAD-autofluorescence, the mitochondrial membrane potential, and the adenine nucleotide content of isolated pancreatic islets and beta cells. 100 μM of moxifloxacin abolished the NAD(P)H increase elicited by 20 mM glucose, while gatifloxacin diminished it and ciprofloxacin had no significant effect. This pattern was also seen with islets from SUR1 Ko mice, which have no functional K_{ATP} channels. Moxifloxacin also diminished the glucose-induced decrease of FAD-fluorescence, which reflects the intramitochondrial production of reducing equivalents. Moxifloxacin, but not ciprofloxacin or gatifloxacin significantly reduced the effect of 20 mM glucose on the ATP/ADP ratio. The mitochondrial hyperpolarization caused by 20 mM glucose was partially antagonized by moxifloxacin, but not by ciprofloxacin or gatifloxacin. Ultrastructural analyses after 20 h tissue culture showed that all three compounds (at 10 and 100 μM) diminished the number of insulin secretory granules and that gatifloxacin and ciprofloxacin, but not moxifloxacin induced fission/fusion configurations of the beta cell mitochondria. In conclusion, fluoroquinolones affect the function of the mitochondria in pancreatic beta cells which may diminish the insulinotropic effect of K_{ATP} channel closure and contribute to the hyperglycaemic episodes.

1. Introduction

During therapy with antibacterial fluoroquinolones, both, hypoglycaemic and hyper-glycaemic episodes are reported to occur (Park-Wyllie et al., 2006). Hypoglycaemias, which occur in most cases during the first three days of therapy, have led to a number of fatalities (Frothingham, 2005). Some of the hypoglycaemic episodes were due to an interaction with insulinotropic antidiabetic drugs, typically sulfonylureas, but there is clear evidence that fluoroquinolones by themselves can increase insulin levels and thus induce hypoglycaemias (Roberge et al., 2000; Menzies et al., 2002; Bhasin et al., 2005).

This has been corroborated in vitro by the demonstration that fluoroquinolones increase insulin secretion by isolated rat pancreatic islets (Maeda et al., 1996). The hypothesis that a block of beta cell K_{ATP} channels was responsible for this effect was confirmed by the demonstration that lomefloxacin and norfloxacin inhibited K_{ATP} channel activity in insulin-secreting RINm5F cells (Zünkler and Wos, 2003). In contrast to sulfonylureas, which act via binding to the regulatory subunit SUR1 (Babenko et al., 1998), the K_{ATP} channel block by fluoroquinolones is due to a direct effect at the pore-forming subunit Kir6.2 (Saraya et al., 2004), similar to the mechanism by which they block cardiac hERG channels (Zünkler et al., 2006). The observation that gatifloxacin and temafloxacin, which were more potent than levofloxacin to block the K_{ATP} channel were also more effective to increase the insulin release from statically incubated mouse islets then led to the conclusion that the hypoglycaemic effect of the fluoroquinolones could be sufficiently explained by this mechanism (Saraya et al., 2004).

In fact, it is currently accepted that the pharmacological closure of K_{ATP} channels is sufficient to stimulate insulin secretion via the depolarization of the beta cell plasma membrane and resultant

Abbreviations: K_{ATP}, channel; ATP, sensitive K^+ channel; NAD(P)H-fluorescence, fluorescence of NADH and NADPH combined; Rh123, Rhodamine 123; SUR, sulfonylurea receptor.

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opening of voltage-dependent Ca^{2+} channels, which in turn activate the exocytotic machinery (Willenborg et al., 2011). However, in a recent study we found that the fluoroquinolones did not stimulate insulin secretion in the presence of a basal glucose concentration, rather, they only enhanced the secretion elicited by a stimulatory glucose concentration (Ghaly et al., 2009). Curiously, it was found in this report that the depolarizing effect was dependent on the cellular model: with open beta cells a depolarizing effect was observed at drug concentrations at which secretion was enhanced, whereas with intact cells much higher concentrations were needed, even though in both cases the drugs were contained in the extracellular solution. This raised the question as to whether fluoroquinolones might exert additional effects on the beta cell which interfere with the depolarization-induced insulin secretion.

For this study three clinically relevant fluoroquinolones were selected: gatifloxacin, ciprofloxacin and moxifloxacin. While case reports of hypoglycaemias exist for a number of fluoroquinolones, pharmacoepidemiologic evidence clearly documents an outstanding role of gatifloxacin as an inducer of hypoglycaemias (Frothingham, 2005; Bhasin et al., 2005; Park-Wyllie et al., 2006). Thus, the use of this compound has been discontinued in spite of its favourable antibacterial properties (Aspinall et al., 2009). Ciprofloxacin has been repeatedly implicated in serious hypoglycaemic episodes, but its safety profile is generally regarded as acceptable (Mohr et al., 2005; Aspinall et al., 2009). Moxifloxacin was originally only rarely implicated in dysglycaemic events (Gavin et al., 2004; Kapoor et al., 2012), however, in a recent cohort study in diabetic patients, the risk of hypoglycaemia was found to be significantly higher than that of ciprofloxacin (Chou et al., 2013).

By studying the effects of these fluoroquinolones on parameters of beta cell energy metabolism we sought to answer the following questions: (i) do the fluoroquinolones have additional sites of action in pancreatic beta cells? (ii) can effects at these sites interfere with the regulation of insulin secretion? and (iii) is there a correlation with the different insulinotropic efficacy of the fluoroquinolones? Answering these questions is not only relevant for the rational further development of fluoroquinolones, it may also be relevant for the understanding of drug-induced insulin secretion in general.

2. Methods

2.1. Chemicals

Gatifloxacin was kindly provided by Grünenthal (Aachen, Germany), ciprofloxacin and moxifloxacin were bought as solutions for i.v. use (Bayer, Leverkusen, Germany). Collagenase NB8 was purchased from Nordmark (Uetersen, Germany), collagen (Type I) from Sigma (Taufkirchen, Germany), cell culture medium RPMI 1640 (without glucose) and fetal calf serum from Gibco/Invitrogen (Karlsruhe, Germany). Rhodamine 123, Luciferase kit for ATP determination and pyruvate kinase were from Sigma. All other reagents of analytical grade were from E. Merck (Darmstadt, Germany).

2.2. Tissue

Islets were isolated from the pancreas of NMRI mice or SUR1 Ko mice (12–16 weeks old, fed ad libitum) by a collagenase digestion technique and hand-picked under a stereomicroscope. Single cells were obtained by incubation of the islets for 10 min in a Ca^{2+}-free medium and subsequent vortex-mixing for 1 min. Islets and single islet cells were cultured on collagen-coated glass cover slips for up to 4 days in cell culture medium RPMI-1640 with 10% fetal calf serum (10 mM glucose until attachment, then 5 mM glucose) in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C.

2.3. Microfluorimetric measurements of NAD(P)H and FAD autofluorescence

Intact islets from NMRI and SUR1 ko mice were cultured on collagen-coated glass cover slips in Petri dishes and were used from day 2–4 after isolation. The cover slip with the attached islet was inserted in a purpose-made perifusion chamber on the stage of an upright epifluorescence microscope (Leitz Orthoplan) equipped with a thermostated (36 °C) objective (Zeiss Fluar 40×, 1.3 N.A.). The islet was perifused with a pre-warmed HEPES-buffered Krebs-Ringer medium, which was saturated with 95% O_2 and 5% CO_2. The NAD(P)H fluorescence was excited at 366 ± 15 nm (Hanau St41 Hg arc lamp), the emission separated by a dichroic mirror at 405 nm and filtered by a 450 ± 32 nm bandpass. The FAD fluorescence was excited at 440 ± 10 nm (Hg arc lamp), the emission separated by a dichroic mirror at 455 nm and a 502 nm longpass filter (filters by Omega Optical, Brattleboro, VT). NAD(P)H or FAD autofluorescence was registered by a photon-counting multiplier (Hamamatsu, Herrsching, Germany) at 1 Hz with 0.1 s exposure time.

2.4. Microfluorimetric measurements of the mitochondrial membrane potential

The mitochondrial membrane potential was measured fluorimetrically using single islet cells which had been cultured for 2–4 days on cover slips. Rhodamine 123 (Rh123) was loaded at a concentration of 10 μM for 15 min. The cover slip with the attached cells was inserted in the same type of perifusion chamber as described above. The fluorescence was excited at 470 ± 20 nm using a 150 W Xenon arc lamp. The fluorescence emission was separated by a 510 nm dichroic and a 520 nm longpass filter and was recorded by a slow-scan CCD camera (TILL Photonics, Gräfelfing, Germany). Upon decrease of the membrane potential this indicator is released from the mitochondria into the cytosol, which leads to a dequenching and thus to an increase in fluorescence (Chen, 1988; Rustenbeck et al., 1997). Whole islets loaded with Rh123 gave slow responses with a very low signal to noise ratio, apparently due to redistribution of the indicator.

2.5. Islet content of adenine nucleotides

15 freshly isolated islets were statically incubated at 37 °C to mimic perifusion conditions. I.e., the islets were incubated for 1 h in the presence of 100 μM of the fluoroquinolones and 5 mM glucose. Then the medium was replaced and after 20 min the glucose concentration was raised from 0 to 20 mM. Thereafter, proteins were precipitated and the adenine nucleotides extracted as described (Urban and Panten, 2005). ATP was determined by use of the luciferase method. The ADP content of the extract was converted into ATP by the pyruvate kinase reaction, the difference between both measurements yielding the net ADP content.

2.6. Electron microscopy

Collagenase-isolated islets from NMRI mice were pooled and cultured in batches of 50 islets. After culturing for 20 h in RPMI 1640 (5 mM glucose) with 10 or 100 μM of either gatifloxacin, moxifloxacin or ciprofloxacin, the islets were fixed for electron microscopy by immersing in a solution of para-formaldehyde (2%) and di-glutaraldehyde (2%) in cacodylate buffer (0.1 M, pH 7.3), post-fixing in osmium tetroxide (1%) for 1 h and embedding in epoxy resin. 50-nm sections of the islets were cut by an