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Disturbance of cellular glucose transport by two prevalently used fluoroquinolone antibiotics ciprofloxacin and levofloxacin involves glucose transporter type 1

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ABSTRACT

Dysglycemia and central nervous system (CNS) complications are the known adverse effects of fluoroquinolone antibiotics. Ciprofloxacin and levofloxacin are among the most prescribed antibiotics. In this study we demonstrate that ciprofloxacin and levofloxacin disturb glucose transport into HepG2 cells and such inhibition is associated with inhibited glucose transporter type 1 (GLUT1) function. When exposed to ciprofloxacin or levofloxacin at maximum plasma concentrations (C_{max}) and 5× of C_{max} concentrations, GLUT1 mRNA expression, cell surface GLUT1 protein expression and glucose uptake were significantly reduced. These findings imply that disturbed cellular glucose transport and GLUT1 function may underlie the dysglycemic and CNS effects of ciprofloxacin and levofloxacin.

1. Introduction

Fluoroquinolones possess a broader spectrum of activity, better pharmacokinetic properties and stronger tissue/cell penetration over other antibiotics. In 2002, fluoroquinolones became the most commonly prescribed class of antibiotics to adults in the United States: 22 million prescriptions, equivalent to 24% of overall prescribed antibiotics, or 106 prescriptions per 1000 adults (Linder et al., 2005). Given such high prescription rates, adverse side effects of fluoroquinolones may pose profound health threats to the general population.

Ciprofloxacin and levofloxacin are the most prescribed fluoroquinolones (Linder et al., 2005). Ciprofloxacin is a second generation fluoroquinolone which was developed in the 1980s (Zhanel et al., 2002). With the expiration of its patent, ciprofloxacin has been manufactured by multiple generic companies (FDA, 2006a). These generic versions will certainly boost the market volume of ciprofloxacin even further. Levofloxacin is a third generation fluoroquinolone with broader spectrum of activity that was introduced in 1997. Since then, the prescriptions of levofloxacin increased rapidly, reaching 8.5 million in 2002, exceeding the 8.1 million prescriptions of ciprofloxacin (Linder et al., 2005).

Dysglycemia and central nervous system (CNS) complications are known adverse effects of ciprofloxacin and levofloxacin. Interaction of ciprofloxacin with glyburide, a hypoglycemic agent, causing persistent hypoglycemia has been reported (Lin et al., 2004; Roberge et al., 2000). Ciprofloxacin has also been reported to inhibit the Na^+-dependent monocarboxylate transport process (Horibe et al., 1998). In addition, levofloxacin was found to be associated with 1.5-fold increased risk of hypoglycemia (Park-Wyllie et al., 2006). In some studies, both agents caused hyperglycemia more frequently than hypoglycemia in diabetic or non-diabetic patients (Coblio et al., 2004). In fact, hyperglycemia may occur with any fluoroquinolone (Mehlhorn and Brown, 2007). On the other hand, CNS effects such as dizziness, headache and seizures are well-known complications during fluoroquinolone therapy (Stahlmann and Lode, 1999). The incidences varied from 0.9% to 3.3% depending on the type of fluoroquinolone (Ball and Tillotson, 1995; Christ, 1990). We have previously observed the disturbance of glucose transporter type 1 (GLUT1) function by gatifloxacin, a fluoroquinolone withdrawn in 2006 because of serious dysglycemic side effects (Ge et al., 2007). The GLUT1 protein is responsible for basal glucose transport into the CNS as well as peripheral tissues (McGowan et al., 1995). Since the effects of ciprofloxacin and levofloxacin on cellular glucose transport remain unknown, in this study we tested the hypothesis that ciprofloxacin and levofloxacin inhibit GLUT1 function and disturb cellular glucose transport.

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2. Materials and methods

2.1. Cell culture

Human hepatoma cells HepG2 (ATCC HB-8065, American Type Culture Collection, Manassas, VA, USA) were maintained at 37 °C in 5% CO_2 in RPMI medium containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco, Invitrogen, Carlsbad, CA, USA). Ciprofloxacin or levofloxacin (Sigma, St. Louis, MO, USA) stock dissolved in phosphate buffered saline (PBS) was mixed with medium to indicated concentrations and applied to cells for 20 h, in the absence of penicillin/streptomycin to prevent drug interaction. Corresponding to the volume of fluoroquinolone stock solution added to the culture media, PBS mixed with media at 0.1% and 0.5% (v/v) ratio were included as the solvent control conditions. Cell viability after ciprofloxacin or levofloxacin treatment at indicated time/concentrations was examined by 1-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT, Sigma) assay and the experimental conditions did not affect cell viability (data not shown).

2.2. RNA extraction, reverse transcription and quantitative real-time PCR

Real-time PCR was carried out as previously described (Ge et al., 2007). Briefly, after fluoroquinolone incubation, total RNA was extracted using TRI reagent (Molecular Research Center, Inc., Cincinnati, OH, USA). Reverse transcription was performed by the MMLV reverse transcriptase system (GE Healthcare, Buckinghamshire, UK). Quantitative real-time PCR was performed by Brilliant SYBR Green QPCR Master Mix (Stratagene, La Jolla, CA, USA) and the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). The expression of the glyceraldehydes-3-phosphate dehydrogenase (GAPDH) gene served as the internal control. The primer information is provided below:

GLUT1	Forward: 5′-CATCAATGCCCCCCAGAA-3′ Reverse: 5′-AAGCGGCCCAGGATCAG-3′ Annealing temperature: 58 °C Amplicon length: 285 bp
GAPDH	Forward: 5′-GGAAGGTGAAGGTCGGAGTC-3′ Reverse: 5′-ACTCCACGACGTACTCAGCG-3′ Annealing temperature: 58 °C Amplicon length: 288 bp

Cycling conditions comprised an initial denaturation of 10 min at 95 °C, followed by 40 cycles with 95 °C denaturation for 30 s, 58 °C annealing for 30 s, and 72 °C elongation for 30 s. The fold change of GLUT1 mRNA content in fluoroquinolone-treated cells relative to that of the 0.1% solvent control treatment was normalized to the internal GAPDH control and analyzed using the $2^{-\Delta\Delta Ct}$ method (Wong et al., 2005a,b).

2.3. Flow cytometry analysis of cell surface GLUT1 protein expression

Surface GLUT1 protein expression was quantified by binding GLUT1 to its ligand, the receptor binding domain of the human T cell leukemia virus (HTLV) envelope glycoprotein (H_{RBD}) fused to enhanced green fluorescence protein (EGFP) (Manel et al., 2003; Montel-Hagen et al., 2008). After fluoroquinolone incubation, the cells were trypsinized and washed with ice-cold PBS. The cell pellet was then resuspended in PBA buffer (PBS with 2% FBS) containing 1:25 dilution of EGFP-conjugated GLUT1 ligand (AbCys S.A., Paris, France) and incubated at 37 °C for 30 min. The cells were then subjected to flow cytometry analysis on a FACSCanto (BD Biosciences, San Jose, CA, USA). The unstained cells served as negative controls and the autofluorescence were subtracted from the total fluorescence of stained cells to give a net fluorescence of EGFP-GLUT1 ligand. The efficacy of EGFP-GLUT1 ligand in staining GLUT1 protein was validated by using differentiating erythroblasts (data not shown) in which surface GLUT1 expression is upregulated during the differentiation process (Montel-Hagen et al., 2008).

2.4. 2-Deoxy-D-glucose (2-DOG) uptake assay

2-DOG uptake assay was carried out as previously described (Ge et al., 2007). Briefly, after fluoroquinolone incubation, cells were washed by PBS for three times to remove the remaining fluoroquinolone and incubated with glucose free Krebs–Ringer phosphate (KRP) buffer at 37 °C for 30 min to deplete intracellular glucose. The subsequent glucose transport study was performed by incubating cells with 700 μl/well hot/cold 2-DOG mixture containing 1 μCi/ml of 2-deoxy-D-[1-^{3}H]-glucose (GE Healthcare) and 0.1 mM of 2-DOG (Sigma) in ice-cold KRP buffer at 37 °C for 5 min. The uptake reactions were stopped by adding 1 ml/well stop solution containing 0.3 mM phloretin (Sigma) in ice-cold PBS followed by washing for twice on ice. Subsequently, cells were lysed and subjected to radioactivity counting by Tri-Carb 2900TR Liquid Scintillation Analyzer (PerkinElmer Life and Analytical Sciences, Waltham, MA, USA). The remaining cell lysates were used to determine protein concentration by the Protein Assay Kit (Bio-Rad, Hercules, CA, USA) for normalizing the amount of glucose influx.

2.5. Statistical analysis

The Student's *t*-test was employed to detect the difference between control and treatment groups. Statistical significance was set at $P < 0.05$ level.

3. Results

3.1. Ciprofloxacin reduces GLUT1 mRNA expression, surface GLUT1 protein expression and glucose transport

The change of GLUT1 mRNA level after 20 h ciprofloxacin incubation was measured by real-time PCR (Fig. 1A). The GLUT1 mRNA content was decreased by 48% and 25% with the treatment of 2.5 μg/ml and 12.5 μg/ml of ciprofloxacin, respectively ($P < 0.05$). Glucose transport assay revealed that the 2-DOG uptake was decreased by 21% and 42% with 20 h ciprofloxacin treatment of

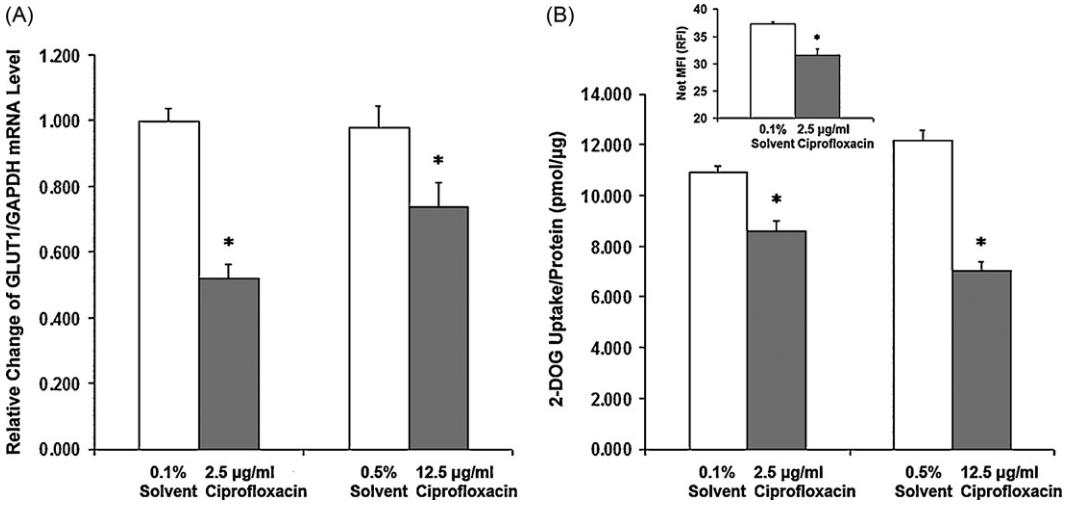

Fig. 1. Ciprofloxacin reduces GLUT1 mRNA expression, surface GLUT1 protein expression and glucose transport. (A) The changes of GLUT1 mRNA content in ciprofloxacin-treated cells were expressed relative to that of the 0.1% solvent control and were normalized to the expression of the internal control gene GAPDH. Data represent mean ± S.E.M. from three independent experiments ($n = 7–11$). (B) The 2-DOG uptake amount was normalized to the total protein content of the cells. Data represent mean ± S.E.M. from three independent experiments performed in triplicate setup ($n = 9$). (B, inset) The mean fluorescence intensity of stained cells with autofluoresence subtracted was expressed as the net mean fluorescence intensity (Net MFI), in units of relative fluorescence intensity (RFI). Data represent mean ± S.E.M. from two independent experiments ($n = 4–6$). Open bars: solvent control. Closed bars: ciprofloxacin. *$P < 0.05$.