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Effect of a dosing-time on quetiapine-induced acute hyperglycemia in mice

Snehal Kapse [a, 1], Hitoshi Ando [a, 1], Yuki Fujiwara [a], Chisato Suzuki [a], Kentaro Ushijima [a], Hiroko Kitamura [a], Keiko Hosohata [a], Kazuhiko Kotani [b], Shigeki Shimba [c], Akio Fujimura [a, *]

[a] Division of Clinical Pharmacology, Department of Pharmacology, School of Medicine, Jichi Medical University, Shimotsuke, Japan
[b] Division of Community and Family Medicine, Center for Community Medicine, Jichi Medical University, Shimotsuke, Japan
[c] Department of Health Science, School of Pharmacy, Nihon University, Funabashi, Japan

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ABSTRACT

Although rare, second-generation antipsychotic drugs cause severe hyperglycemia within several days after the initiation of therapy. Because glucose tolerance exhibits circadian rhythmicity, we evaluated an effect of a dosing-time on quetiapine-induced acute hyperglycemia in mice. A single intraperitoneal dose of quetiapine dosing-time-independently induced insulin resistance in fasted C57BL/6J mice. However, acute hyperglycemic effect was detected only after dosing of the drug at the beginning of an active phase. Under the conditions in which hepatic glucose production was stimulated by pyruvate administration, hyperglycemic effect of quetiapine was dosing-time-independently observed. In addition, the dosing-time-dependent hyperglycemic effect of quetiapine disappeared in the liver-specific circadian clock-disrupted mice in which circadian rhythmicity in hepatic glucose production is deranged. Furthermore, the dosing-time had little impact on the pharmacokinetics of quetiapine in normal mice. These results suggest that quetiapine acutely causes hyperglycemia only when hepatic glucose production elevates. Therefore, quetiapine therapy with once daily dosing at a rest phase might be safer than that at an active phase. Further studies are needed to confirm the hypothesis.

1. Introduction

Second-generation antipsychotics (SGAs) (also known as atypical antipsychotics) are the current standard drugs for the treatment of schizophrenia, because their tolerances are generally better than those of older antipsychotics (1). However, SGAs frequently lead to adverse metabolic outcomes, including weight gain and hyperglycemia (1,2). As a result, severe or life-threatening adverse effects such as diabetic ketoacidosis can occur in a subset of patients (3,4). Although SGA-induced overweight/obesity is often involved in the development and/or exacerbation of diabetes, there is strong evidence that SGAs cause glucose intolerance without weight gain (1,2). Diabetic ketoacidosis preceded weight gain in over one third of cases, and was diagnosed within 1 week after the initiation of SGA therapy in some patients (3). Therefore, the preventive strategies against SGA-associated hyperglycemia are required for rational SGA therapy. At present, only way might be to avoid the administration of SGAs to patients with diabetes, although most cases of SGA-induced diabetic ketoacidosis did not have overt diabetes prior to taking an SGA (3).

The precise mechanisms underlying the hyperglycemic effect of SGAs remain unclear. *In vitro* studies have shown that SGAs (including quetiapine and clozapine) inhibit glucose uptake in neuronal PC12 cells after an incubation for a short period (30 min), possibly by direct binding to the glucose transporter (GLUT) (2,5). A single dose of these SGAs could induce hyperglycemia within 30 min after administration in mice, and the elevated blood glucose levels were significantly correlated with their *in vitro* ability to inhibit glucose transport (6). These results suggest that the blockade of GLUT is involved in the mechanisms by which SGAs acutely cause hyperglycemia.

* Corresponding author. Department of Pharmacology, School of Medicine, Jichi Medical University, Shimotsuke, Tochigi 329-0498, Japan. Fax: +81 285 44 7562.
E-mail address: akiofuji@jichi.ac.jp (A. Fujimura).

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[1] The first two authors contributed equally to this study.

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Fasting blood glucose (FBG) concentrations exhibit daily rhythmicity with a peak at the beginning of an active phase in animals (7,8). An early morning increase in FBG is also detected in humans (9,10). The fluctuation in FBG is consistent with the circadian rhythms of hepatic gluconeogenesis and glucose production observed in animals (11,12) and humans (13), respectively. Glucose uptake exhibits a similar rhythm in rats (14), and more glucose is taken up early in the morning compared to that in the evening in humans (15,16). Thus, both glucose production and usage elevate at the beginning of an active phase in both animals and humans. Taken together, these findings provide the possibility that a dosing-time of SGAs influences their acute hyperglycemic effect. To address the hypothesis, we evaluated a dosing-time-dependent effect of quetiapine on FBG concentrations in mice.

2. Materials and methods

2.1. Animals

The protocol of the study was approved by the Institutional Animal Experiment Committee of Jichi Medical University (Shimotsuke, Japan). All animal procedures were performed in accordance with the Institutional Regulation for Animal Experiments and Fundamental Guideline for Proper Conduct of Animal Experiment and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology of Japan. All efforts were made to minimize animal suffering.

Seven- to eight-week-old male C57BL/6J mice were purchased from Charles River Japan (Yokohama, Japan). C57BL/6J-background, liver-specific *Bmal1*-deficient (L-*Bmal1*−/−) and control (flox/flox) mice (7, 17) homozygous for floxed *Bmal1*, with and without Cre driven by the albumin promoter, respectively, were bred and raised at the Center for Experimental Medicine of Jichi Medical University. All mice were maintained under specific pathogen-free conditions and controlled temperature and humidity with a 12/12 h light/dark cycle and fed a regular diet (CE-2; CLEA Japan, Tokyo, Japan) and water *ad libitum*.

2.2. Experiments

Mice were fasted for 20 h and then administered saline i.p. containing 1.25% dimethyl sulfoxide with or without 10 mg/kg quetiapine fumarate (Toronto Research Chemicals, Toronto, Canada). Another subset of mice was fasted 20 h and thereafter given saline i.p. containing 0.5% acetic acid with or without 20 mg/kg aripiprazole (Wako, Osaka, Japan). All mice were not fed during the course of the experiments. The dosages of quetiapine and aripiprazole were chosen on the basis of data from the previous studies in mice (6,18). Zeitgeber time (ZT) was used to describe the experimental time with ZT 0 defined as lights on and ZT 12 as lights off. To reduce the number of mice, a cross-over design with a 14-day interval was used in all experiments other than experiment 4, as described below.

2.2.1. Experiment 1: A dosing-time-dependent effect of quetiapine on FBG concentrations

Ten-week-old C57BL/6J mice ($n = 32$) were administered quetiapine or vehicle at ZT 2, 8, 14, or 20 ($n = 4$ in each condition). At 12 weeks of age, mice were given the other treatment at same ZT. Glucose concentrations in blood (taken from the tail) were measured at 0, 0.5, 1, 2, and 3 h after the administration using a Glutest Ace R (Sanwa Kagaku Kenkyusyo, Nagoya, Japan).

In a similar way, 10-week-old male L-*Bmal1*−/− ($n = 14$) and flox/flox mice ($n = 16$) were administered quetiapine or vehicle at ZT 2 or 14, and 2 weeks later, the other treatment by a cross-over design. Moreover, 10-week-old C57BL/6J mice ($n = 16$) were given aripiprazole or vehicle at ZT 2 or 14 in the same fashion.

2.2.2. Experiment 2: An effect of quetiapine on FBG concentrations during insulin tolerance test

At 10 and 12 weeks of age, C57BL/6J mice ($n = 16$) were administered quetiapine or vehicle (in a cross-over fashion) at ZT 2 or 14 ($n = 8$ in each ZT). One hour after administration, insulin (Novolin® R, Novo Nordisk Pharma, Tokyo, Japan) was injected i.p. at 0.5 U/kg. Blood glucose concentrations were determined at 0, 0.5, 1, and 2 h after insulin challenge.

2.2.3. Experiment 3: An effect of quetiapine on FBG concentrations during pyruvate challenge test

At 10 and 12 weeks of age, C57BL/6J mice ($n = 16$) were given quetiapine or vehicle (in a cross-over fashion) at ZT 2 or 14 ($n = 8$ in each ZT). One hour after dosing, sodium pyruvate (Sigma−Aldrich, St. Louis, MO) was injected i.p. at 2 g/kg. Blood glucose concentrations were measured at 0, 0.5, 1, 2, and 3 h after pyruvate challenge.

2.2.4. Experiment 4: An effect of quetiapine on hepatic mRNA expression of glucose production-related genes

Ten-week-old C57BL/6J mice ($n = 16$) were administered quetiapine or vehicle at ZT 2 or 14 ($n = 4$ in each condition). One and a half hours after the administration, mice were anesthetized with pentobarbital (30 mg/kg, i.p.) and sacrificed to obtain blood and liver samples. Blood was mixed immediately with EDTA and aprotinin and centrifuged at 4 °C to separate plasma. The liver samples were directly placed into RNAlater® solution (Thermo Fisher Scientific, Waltham, MA). These samples were stored at −80 °C until assayed.

2.2.5. Experiment 5: A dosing-time-dependent effect on plasma quetiapine concentration

Ten-week-old C57BL/6J mice ($n = 16$) were administered quetiapine at ZT 2 or 14. At 1 and 3 h after the administration, mice were anesthetized and sacrificed to obtain blood samples for measuring plasma quetiapine concentrations.

2.3. Plasma insulin and glucagon measurement

Plasma concentrations of insulin and glucagon were measured using MILLIPLEX® MAP mouse metabolic magnetic bead panel kit (Merck KGaA, Darmstadt, Germany), according to the manufacturer's instructions. Data were obtained using the MAGPIX® system with xPONENT 4.2 software (Merck KGaA). Lower limits of quantification of insulin and glucagon were 69 and 14 pg/ml, respectively. The intra-and inter-assay coefficients of variation were all less than 15%.

2.4. RNA extraction and real-time quantitative PCR

Total RNA was isolated from the liver samples using a PureLink® RNA Mini Kit (Thermo Fisher Scientific) and reverse-transcribed using a PrimeScript RT reagent Kit (Takara Bio, Otsu, Japan). Gene expression was analyzed using real-time quantitative PCR with the Applied Biosystems StepOnePlus Real-Time PCR System (Thermo Fisher Scientific). Specific sets of primers and TaqMan probes (TaqMan Gene Expression Assays) were obtained from Thermo Fisher Scientific. To control for variation in the amount of cDNA available for PCR in the different samples, expression of target sequences was normalized to an endogenous control, glyceraldehyde-3-phosphate dehydrogenase (*Gapdh*). The GenBank accession

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