Clozapine-Induced Acute Hyperglycemia Is Accompanied with Elevated Serum Concentrations of Adrenaline and Glucagon in Rats

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Clozapine, an atypical antipsychotic agent, has been reported to cause acute hyperglycemia. However, the mechanism of clozapine-induced rapidly developing hyperglycemia is not well elucidated. To clarify the mechanism underlying clozapine-induced acute hyperglycemia, we investigated the effects of single intravenous administration of clozapine on the serum concentrations of glucose and several endogenous substances in rats. Male Wistar rats received an intravenous injection of saline (control) or clozapine 2.5, 5, 10 mg/kg. Blood samples were obtained periodically after clozapine administration to determine the serum concentrations of glucose, adrenaline, glucagon, insulin, corticosterone, and clozapine. The serum concentrations of glucose, adrenaline, and glucagon increased dose-dependently after the administration of clozapine at 2.5–10 mg/kg, and reached maxima at 5 mg/kg of clozapine. The serum concentration of corticosterone increased after the administration of clozapine, but no significant variation was observed with the dosage of clozapine. The concentrations of serum insulin increased in a dose-dependent manner after clozapine administration. In conclusion, a single administration of clozapine increased the serum concentration of glucose in rats, and adrenaline and/or glucagon would be associated with clozapine-induced acute hyperglycemia.

Key words clozapine; hyperglycemia; adrenaline; glucagon; rat

Dysglycemia refers to abnormal blood sugar levels from any cause and is associated with increased morbidity and mortality.1,2) Treatments involving gatifloxacin3) (a novel fluoroquinolone antimicrobial drug) and olanzapine4) and quetiapine5) (atypical antipsychotic drugs) were previously found to induce severe dysglycemia; an Emergency Safety Information statement was issued in Japan, and the contraindication of these drugs for patients with diabetes melitus was added to package inserts. Ultimately, the oral dosage form of gatifloxacin was withdrawn from the market.

We previously reported that novel quinolone antimicrobial agents such as gatifloxacin, levofloxacin, and moxifloxacin increased serum glucose concentrations in rats, in association with the induction of histamine release by these drugs, leading to elevations in serum adrenaline concentrations.6–9) In contrast, a single intravenous injection of olanzapine increased the serum glucose and adrenaline levels without histamine release.10) These results suggest that the mechanisms responsible for drug-induced elevations in serum glucose levels vary among these drugs.

Clozapine is one of the first effective antipsychotic agents to have few of the extrapyramidal side effects typical of nearly all the antipsychotic agents in clinical use.11) However, abnormalities of glucose regulation during clozapine treatment have been reported, similarly to olanzapine. Although clozapine was administered chronically in many of these cases,12–16) there is also a case report in which a patient developed rapid-onset hyperglycemia immediately after starting clozapine.17)

Previous studies have attempted to elucidate the mechanism of hyperglycemia in chronic administration of clozapine18); it may be associated with induction of insulin resistance by directly impairing insulin-responsive glucose resistance in adipocytes.19) There are, in contrast, few reports about the mechanism of hyperglycemia by acute administration of clozapine. Although Smith et al. showed that clozapine-induced glucagon secretion from pancreatic islets was partly associated with the induction of an increase in serum glucose concentration,20,21) it has not been sufficiently elucidated whether clozapine affects various endogenous substances associated with glucose homeostasis.

In the present study, we investigated the effects of a single intravenous injection of clozapine on the serum levels of glucose and several endogenous substances including adrenaline, corticosterone and glucagon, in rats.

MATERIALS AND METHODS

Animals Male Wistar rats (Japan SLC, Hamamatsu, Japan) weighing 235–270 g were used. Rats were housed in a controlled environment and fasted overnight before the experiment. Rats had indwelling cannulas implanted in the left carotid artery and jugular vein under isoflurane anesthesia for blood sampling and intravenous injections, respectively. Animal experiments were performed in accordance with the Guidelines for Animal Experiments of Tokyo Medical and Dental University.

Materials Clozapine was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Regarding the intravenous administration, 25 mg of clozapine was dissolved in

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1.9 mL of 0.1 M hydrochloric acid and the solution was adjusted to 1, 2.5, and 5 mg/mL with saline. α-Hydroxy-midazolam (as an internal standard for the assay of clozapine), (R)-(−)-epinephrine, histamine dihydrochloride, and betamethasone (as an internal standard for the assay of corticosterone) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) Corticosterone and potassium ferricyanide (III) were from Sigma-Aldrich (St. Louis, MO, U.S.A.). α-Phthalaldehyde was purchased from Nacalai Tesque Inc. (Kyoto, Japan). (1R,2R)-(−)-1,2-Diphenylethylenediamine was purchased from Tokyo Chemical Industry Co., Ltd. All chemicals were of analytical grade.

**Single Intravenous Administration Study of Clozapine in Rats** Clozapine at 2.5, 5, and 10 mg/kg was intravenously injected for 30 s to rats at least 1 h after emerging from the anesthesia. Blood samples (650 μL) were obtained 0, 15, 30, 60, 120 and 240 min after the clozapine injection to determine the serum concentrations of glucose, adrenaline, glucagon, insulin, corticosterone, and clozapine. An equivalent volume of normal saline was injected in rats as control.

In the second set of rats, clozapine at 5 mg/kg or saline was intravenously injected to rats in the same way as the first set, and blood samples (500 μL) were obtained 0, 15, 30 and 60 min after the clozapine injection to determine the serum concentrations of glucose and histamine.

In these experiments, clozapine or saline was administered from 11:00 a.m. to 1:00 p.m.

**Analytical Methods** Serum concentration of glucagon was determined by an enzyme immunoassay using Mercodia Glucagon ELISA-10 μL (Mercodia, Uppsala, Sweden). Serum concentration of clozapine was determined by a slight modification of HPLC assay of Ma and Lau.22) Clozapine was detected with an UV detector set at a wavelength of 230 nm, and the calibration curve for clozapine was linear within the concentration range of 25–1000 ng/mL. The HPLC apparatus was a LC-20AD (Shimadzu Co., Kyoto, Japan) equipped with an UV detector (SPD-20A, Shimadzu Co.) and a fluorescence detector (RF-20Axs, Shimadzu Co.). The column was TSK-gel ODS-80TM (5 μm, 4.6 mm i.d.×15 cm, TOSOH, Japan) and was kept at 40°C. Serum concentrations of other substances (glucose, adrenaline, corticosterone, insulin, and histamine) were determined as previously described.23)

**Pharmacokinetic Analysis** Pharmacokinetic analysis was performed using the non-linear mixed-effects modeling approach in NONMEM (v7.3.0, ICON Development Solutions, Hanover, MD, U.S.A.) with first-order conditional estimation with interaction (FOCE-INTER). The subroutines ADVAN3 and TRANSI were used to evaluate the two-compartment pharmacokinetic model. The model was parameterized in terms of total body clearance ($CL_{\text{tot}}$), distribution volume of the central compartment ($V_1$), and the first-order transfer rate constants between the central and peripheral compartments ($k_{12}/k_{21}$). The inter-individual variability of the pharmacokinetic parameters was estimated using an exponential error model. The intra-individual variability of the serum concentration of clozapine was estimated using the proportional error model.

**Statistical Methods** All data represent the mean±standard deviation (S.D.). Statistical evaluations were performed using Student’s t test or Tukey–Kramer multiple comparison test. Differences were considered significant at $p<0.05$.

**RESULTS**

**Pharmacokinetics of Clozapine in Rats** The time profiles of serum concentrations of clozapine after single intravenous injection of clozapine at a dose of 2.5, 5, or 10 mg/kg in rats are shown in Fig. 1. The data were fitted to the two-compartment open model and the estimated population values of $CL_{\text{tot}}$, $V_1$, $k_{12}$, and $k_{21}$ were 0.111±0.021 L/min/kg, 8.84±1.12 L/kg, 0.00799±0.00313/min, and 0.00655±0.00625/min, respectively. The mean inter-individual variabilities of $CL_{\text{tot}}$, $V_1$, $k_{12}$, and $k_{21}$ were 11.5, 12.0, 32.0, and 72.0%, respectively. The intra-individual variability of the serum concentration of clozapine was 5.44%. Simulation curves for serum concentration of clozapine drawn by these population parameters are shown in Fig. 1. These simulation curves matched well with the data obtained.

**Clozapine Effects in Rats** The serum concentrations of glucose, adrenaline, and glucagon increased dose-dependently...
after clozapine administration (2.5–10 mg/kg), and the elevations in these concentrations reached maxima at 5 mg/kg of clozapine (Figs. 2, 3A, B). The serum concentration of corticosterone increased after the injection of clozapine, but there was no statistically significant difference among the different clozapine dose groups (Fig. 3C). The concentrations of serum insulin increased dose-dependently after clozapine administration (Fig. 3D).

In a separate experiment, clozapine (5 mg/kg) or saline was administered to rats and serum concentrations of glucose and histamine were determined. Clozapine induced elevation of serum glucose was confirmed (Fig. 4A) and serum histamine concentrations remained unchanged (Fig. 4B).

DISCUSSION

In the present study, the serum concentrations of glucose increased after the injection of clozapine. This result suggests that rats are an appropriate model for investigating clozapine-induced acute hyperglycemia in humans. Since the serum concentrations of clozapine observed in rats included the therapeutic range of clozapine in humans (350–600 ng/mL), the blood glucose level of patients treated with clozapine should be carefully monitored.
Several endogenous compounds, including catecholamines, glucagon and glucocorticoids, are recognized as factors that regulate the serum concentration of glucose. In an attempt to clarify the mechanism of clozapine-induced acute hyperglycemia, we investigated the effects of a single injection of clozapine on the serum levels of these endogenous substances. In the present study, the serum concentrations of adrenaline and glucagon increased after the administration of clozapine. Our results suggest that adrenaline and/or glucagon would be associated with clozapine-induced hyperglycemia.

Our previous reports demonstrated that fluoroquinolone antimicrobial agents can induce histamine release leading to an increase in serum epinephrine concentrations and hyperglycemia. In contrast, a single intravenous injection of clozapine increased the serum glucose levels without histamine release. Olanzapine also induced hyperglycemia without affecting the serum concentration of histamine. These results suggest that the mechanisms responsible for drug-induced hyperglycemia may be different between atypical antipsychotics and fluoroquinolones.

We previously reported that olanzapine-induced elevation of serum glucose concentration was suppressed completely by pretreatment with propranolol (β-adrenergic antagonist). In addition, the administration of olanzapine did not affect the serum concentration of glucagon. Therefore, adrenaline plays a major role in olanzapine-induced acute hyperglycemia. Compared to our previous study using olanzapine, not only adrenaline but also glucagon increased significantly following a single administration of clozapine. Adrenaline was known to increase serum glucagon levels. However, the increase of adrenaline levels in olanzapine-treated rats did not affect the serum concentration of glucagon. Therefore, the increase of serum glucagon levels in rats with clozapine treatment in this study would not be due to the increase of adrenaline. Smith et al. previously reported that glucagon levels were higher in rats following a clozapine injection, and that this increase was the major contributor to elevated blood glucose levels. These results suggest that not only adrenaline but also glucagon is associated with clozapine-induced elevations in blood glucose levels, and the mechanism responsible for the induction of hyperglycemia following a single injection of clozapine may differ from that by olanzapine.

Clozapine is categorized as multi-acting receptor targeted antipsychotics and shows an affinity for dopamine, adrenergic, histamine, muscarinic and serotoninergic receptors. Ikegami et al. reported that antagonizing histamine H1 receptors, dopamine D2 receptors and α1-adrenoceptors induced hyperglycemia after intracerebroventricular administration of each antagonists. Therefore, clozapine-induced hyperglycemia may be associated with antagonizing these receptors in the brain. On the other hand, the affinity of clozapine for several receptors differs from that of olanzapine. Binding affinities of olanzapine for D2, D3, and 5-hydroxytryptamine type 2A (5-HT2A) receptors were higher than those of clozapine. In contrast, the affinities of clozapine for α1, α2, 5-HT1A, 5-HT2B, and 5-HT7 receptors were higher than those of olanzapine. These differences may be associated with the different mechanisms responsible for drug-induced hyperglycemia.

Glucocorticoids induce hyperglycemia through gluconeogenesis by increasing the rate of transcription of genes that encode gluconeogenic enzymes and increasing peripheral insulin resistance. In the present study, the serum concentration of corticosterone increased after the administration of clozapine, but with no significant difference for different doses of clozapine. This result was almost the same as our previous study using olanzapine, and suggests that corticosterone was not a major factor in the clozapine-induced increase in serum glucose concentration.

In the present study, the concentration of serum insulin increased in a dose-dependent manner after clozapine administration, whereas the elevation in glucose concentration reached a maximum at 5 mg/kg of clozapine. Smith et al. reported in their study of isolated mouse islets that clozapine significantly increased glucose-stimulated insulin secretion. These results suggested that the increase in insulin levels in our study would be attributed not only to the rise in serum glucose but also to the direct effect of clozapine on insulin secretion.

In conclusion, a single intravenous administration of clozapine increased the serum concentration of glucose in rats, and adrenaline and/or glucagon would be associated with clozapine-induced acute hyperglycemia.

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Conflict of Interest The authors declare no conflict of interest.

REFERENCES


