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ORIGINAL RESEARCH

Computational and Experimental Investigation of Antidiabetic Drugs on Tofacitinib Metabolism: Molecular Docking, in vitro, and in vivo Studies

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Background: Tofacitinib is an orally administered Janus kinase (JAK) inhibitor that has demonstrated significant efficacy in the treatment of rheumatoid arthritis. This study aimed to investigate the effects of gliquidone and linagliptin, two hypoglycemic agents on the pharmacokinetics of tofacitinib in vitro and in vivo.

Methods: The mechanism of drug-drug interaction was studied in vitro using a murine liver microsome incubation system and in vivo by administering gliquidone and linagliptin orally to rats pretreated with various concentrations of tofacitinib. This study used waters ACQUITY UPLC I-Class/Xevo TQD ultra-high performance liquid chromatography-tandem triple quadrupole mass spectrometer. Furthermore, molecular docking was performed to simulate the interaction using computer simulations.

Results: Gliquidone and linagliptin inhibited the metabolism of tofacitinib by heparanase in vitro with IC_{50} values of 1.140 μM and 4.064 μM, respectively. Co-administration of gliquidone significantly increased the $AUC_{(0-t)}$ of tofacitinib by approximately 43.3%, accompanied by a 45.1% increase in C_{max} and a 27.5% reduction in clearance (CLz/F). In contrast, linagliptin exhibited a more potent inhibitory effect, raising the $AUC_{(0-t)}$ approximately 4.4-fold, enhancing the C_{max} by 2.86-fold, and decreasing clearance to 25.8% of the control level. These findings suggest that while both gliquidone and linagliptin significantly enhance the systemic exposure of tofacitinib, linagliptin demonstrates a markedly more significant inhibitory effect on tofacitinib's metabolism and elimination.

Conclusion: Gliquidone and linagliptin significantly altered the pharmacokinetics of tofacitinib in vitro and in vivo. This study demonstrated the drug-drug interactions between linagliptin, gliquidone, and tofacitinib, highlighting the need for clinical attention to this possibility.

Keywords: antidiabetic drugs, tofacitinib, drug-drug interaction, pharmacokinetics, molecular docking

Introduction

Rheumatoid arthritis (RA) is an autoimmune disease characterized by excessive inflammation of tendons (tenosynovitis), the proliferation of synovium in joints and the destruction of cartilage, accompanied by several systemic complications and early death.[1] The quality of life of the patient is negatively affected by this condition.[2] However, current data reports that the prevalence of RA ranges from 0.21% to 1% among individuals between the ages of 20 and 40.[3]

The drug tofacitinib is safe and effective for RA and is designed for the treatment of adult patients with moderate to severe active RA who are intolerant to methotrexate.[4] Several studies have demonstrated that tofacitinib attenuates the production of inflammatory mediators and interferes with sensors of Janus kinases (JAK) signaling and transcriptional activator (STAT) pathways.[5,6] Research studies and real-life data have revealed that some oral Janus kinase inhibitors

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(JAKi), including tofacitinib and baricitinib, have rapid pharmacologic actions and can be combined with other antirheumatic drugs (DMARDs) to enhance drug efficacy.[7] Furthermore, JAK inhibitor is relatively easy to produce and tofacitinib is usually the preferred therapy for moderate to severe RA patients.[8] Approximately 70% of the tofacitinib is metabolized in the liver, primarily through oxidative and N-demethylation pathways. Specifically, approximately 50% processed by cytochrome P450 3A4 (CYP3A4) and 20% processed by cytochrome P450 2C19 (CYP2C19).[9] Ketoconazole combined with tofacitinib increases the value of the area under the plasma concentration-time curve (AUC) by 103% and the maximum plasma concentrations (C_{max}) by 16% in healthy male adults. Fluconazole may enhance the AUC and C_{max} of tofacitinib by 79% and 27%, respectively, when combined with tofacitinib.[10] Metabolism-based inhibition of CYP3A4 may result in clinically significant interactions with the drug.[11,12] Therefore, the interaction between tofacitinib and other medications should be closely monitored.

Diabetes Mellitus (DM) is one of the fastest-growing metabolic diseases of the 21st century due to the increasing global burden of chronic diseases. DM is expected to affect approximately 537 million adults by 2021, with more than 90% of cases being Type 2 Diabetes Mellitus (T2DM). Moreover, the number of affected adults is estimated to reach 643 million by 2030.[13,14] The majority of people with T2DM require multiple therapies to achieve treatment goals and prevent complications.[15] T2DM patients are frequently associated with chronic low-grade systemic inflammation, which is increasingly recognized as an inflammatory disease.[16] Notably, studies revealed an increased prevalence of DM in patients with RA compared to controls (OR=1.74, 95% CI:1.22–2.50),[17] prompting the exploration of anti-inflammatory therapies for dual management of T2DM and RA. Studies have shown that specific anti-inflammatory agents may be beneficial for the treatment of both T2DM and RA.[18] The Mendelian randomization study further indicated that thiazolidinedione can significantly lower the risk of RA.[19] Additionally, T2DM affects over 25% of elderly patients aged 65 and older.[20] One study found that 29.4% of elderly patients take six or more medications regularly.[21] Due to older patients' vulnerability to complications and multiple chronic conditions, and as polypharmacy increases, the risk of drug-drug interactions (DDIs) also rises, making careful medication management essential.[22,23]

Linagliptin and gliquidone are commonly used to treat T2DM.[24] Gliquidone, designated by WHO as the sulfonylurea of choice for patients with mild to moderate renal insufficiency,[24] is a widely used oral antidiabetic drug with few adverse effects.[23] It is rapidly absorbed after oral administration and has a short half-life of approximately 1.5 hours. Human recombinant enzyme studies have shown that CYP3A4 is the primary enzyme for gliquidone metabolism. The highest metabolizing capacity for gliquidone was demonstrated by CYP3A4.[25] Linagliptin is a selective inhibitor of dipeptidyl peptidase (DPP-4). The active glucagon-like peptide-1 effectively inhibitor the synthesis of cytochrome P450 (CYP) enzyme.[26,27] Gastric inhibitory peptides and active glucagon-like peptide-1 (GLP-1) lower glucose levels.[28] Furthermore, linagliptin was found to inhibit the activity of CYP3A4[29] moderately. Linagliptin is a substrate for human CYP3A4 enzymes and P-glycoproteins (P-gps) and has a lower bioavailability of approximately 30%.[30] Researchers found that patients with T2DM taking DPP4 inhibitors also had a lower risk of RA compared to those not taking them.[31] The American Diabetes Association and the American Geriatrics Society suggest that DPP-4 inhibitors may be beneficial to elderly patients.[27] Many drugs exert their pharmacological effects through the mechanism of CYP3A4 inhibitors. Accumulating evidence indicates that the inhibition of CYP3A4 reduces the initial clearance of drugs in the liver and intestine, thereby altering pharmacokinetic behavior and potentially improving therapeutic effectiveness.[12] Therefore, special consideration should be given to the interactions between gliquidone and linagliptin.

Gliquidone and linagliptin are commonly prescribed drugs for the treatment of patients with DM and RA, which often co-exist. Gliquidone and linagliptin may interact with tofacitinib when diabetic patients receive long-term therapy. Previous studies suggest that the dose of tofacitinib should be adjusted when administered with CYP3A4 inhibitors of moderate potency.[32] No data have been published regarding the interactions between tofacitinib, gliquidone, and linagliptin. However, drug-drug interaction is anticipated.

This study aimed to investigate the effects of gliquidone and linagliptin, two hypoglycemic agents, on the pharmacokinetics of tofacitinib in vitro and in vivo. The mechanism of drug-drug interaction was studied in vitro using a murine liver microsome incubation system and in vivo by administering gliquidone and linagliptin orally to rats pretreated with various concentrations of tofacitinib. Molecular docking was performed to simulate the interaction using computer

simulations. In summary, our research can provide a valuable reference for the design of a more effective and safe treatment program for patients with co-existing RA and DM.

Materials and Methods

Chemicals and Reagents

Tofacitinib (purity ≧98%); midazolam (1 mg/mL) was purchased from Jiangsu Enhua Pharmaceutical Co, Ltd, Gliquidone (purity >98%, Macklin), Linagliptin (purity >98%, Macklin) and Tofacitinib (purity >98%, Macklin), Glimepiride (purity >98%, Macklin), Repaglinide (purity >98%, Macklin), Empagliflozin (purity >98%, Macklin), Tolbutamide (purity >98%, Macklin), Metformin (purity >98% Macklin), Acarbose (purity >98%, Macklin), Probucol (purity >98%, Macklin), Acipimox (purity >98%, Macklin), Clofibrate (purity >98%, Macklin), CholestyraMine (purity >98%, Aladdin). (purity>98%, Macklin), Clofibrate (purity>98%, Macklin) and CholestyraMine (purity>98%, Aladdin) were purchased from Beijing Inokai Science and Technology Co. Ltd; methanol (chromatography grade, Merck), acetonitrile (chromatography grade, Merck) were purchased from Shanghai Sigma Aldrich Trading Co. Ltd; NADPH was purchased from Roche and the experimental water was purified by the Hetai Smart-Q15 Water Purification System.

Ethical Statement and Animal Experiments

Sprague-Dawley (SD) rats, weighing approximately 220 ± 10 g, were obtained from the Experimental Animal Center of Wenzhou Medical University. The license number for rats is SCXK (Zhejiang) 2020 0001, while the usage license number is SYXK (Zhejiang) 2020 0014. SD rats are housed in habitats maintained at 22 °C and 70% relative humidity. Food and water were provided ad libitum, and the air conditioner maintained a comfortable temperature. The corncob bedding was changed every week. During the first week of their acclimatization, the rats were used for experiments and fasted overnight before being examined. The guidelines for the care and use of laboratory animals, the Animal Welfare Act and the Office of Laboratory Animal Welfare were followed during the conduct of all animal experiments. The Animal Experimental Ethics Inspection Department of the Laboratory Animal Center at Wenzhou Medical University reviewed and approved all the experimental procedures (approval no. wydw2019-650).

Instruments and UPLC-MS/MS Conditions

Waters ACQUITY UPLC I-Class/Xevo TQD ultra-high performance liquid chromatography-tandem triple quadrupole mass spectrometer was performed in the experiment. Experimental and instrumental parameters were optimized to achieve the best detection.

In this experiment, we used a binary pump with acetonitrile as mobile phase A and 0.1% formic acid aqueous solution as mobile phase B (pH about 3). The flow rate for the separation of tofacitinib and the internal standards was set to 0.4 mL/min using an HSS T3 chromatographic column. We used gradient elution to remove the chromates and the injection capacity was 2 μL. Approximately 3 min are devoted to the performance. The ratios for the initial flow were 10% mobile phase A and 90% mobile phase B. During the 0–0.5 min period, the mobile phase A concentration was increased to 30%. Between 0.5–1 min, the concentration of mobile phase A was increased to 95%. At 1–2 min and 2–2.3 min, 10% of mobile phase A was maintained, and the ratio was thendecreased to 10% of mobile phase A.

The conditions of mass spectrometry detection were quantitatively examined using positive ion mode and multiple reaction monitoring (MRM). We measured the capillary voltage, the dissolvent gas temperature, the source temperature, the sheath gas flow rate and the dissolvent gas flow rate at 3 kV. The monitoring ion pair of tofacitinib was m/z 313.2 → m/z 149.0, the cone voltage was 40V and the collision energy was 30V. The monitoring ion pair of tofacitinib metabolite M8 was m/z 299.2 → m/z 98.1, the cone voltage was 40V and the collision energy was 30V. The monitoring ion pair of midazolam (IS) was m/z 326.0 and m/z 291.1, the cone voltage was 50 volts and the collision energy was 26 volts.

Preparation and Quantification of Protein Concentration of RLMs

The rat liver microsomes were prepared in our laboratory using a previously described method.[27] The rats were fully anesthetized with 0.6% pentobarbital sodium (0.65 mL/100g), dissected and removed from the liver and then perfused with physiological saline (0.9%). The liver tissues were accurately weighed, homogenized under ice bath conditions and centrifuged for 30 min at 4°C. The supernatants were transferred to a new centrifuge tube and centrifuged for 60 min at 4°C. The supernatant of the centrifuged samples was then removed and the precipitates were resuspended in 0.15 mol KCl PBS solution containing 0.25 mol sucrose. Microsomes prepared from the rat liver were packaged and stored in a refrigerator at −80°C for future use. The environment was maintained at 4°C to prevent enzyme inactivation. The concentration of proteins was determined by using the BCA protein assay kit.

Methodology Validation

A rigorous validation of the UPLC-MS/MS method was performed in accordance with FDA guidelines, assessing linearity, precision, accuracy, and stability. Our previous study describes the validation procedures with detailed methodology and evaluation criteria.[33,34]

In vitro Microsomal Screening

To evaluate the effects of commonly used antidiabetic drugs on the metabolism of tofacitinib, an in vitro microsomal incubation assay was performed. A total of 12 antidiabetic drugs, including Gliquidone, Linagliptin, Glimepiride, Repaglinide, Empagliflozin, Tolbutamide, Metformin, Acarbose, Probucol, Acipimox, Clofibrate, and Cholestyramine, were prepared at a final concentration of 100 μM. The incubation system (200 μL) consisted of 0.3 mg/mL human liver microsomes, 40 μM tofacitinib, 100 mm potassium phosphate buffer (pH 7.4), and each of the tested antidiabetic drugs. The mixture was pre-incubated at 37°C in a shaking water bath for 5 min before initiating the reaction by adding the NADPH-generating system. The reaction was allowed to proceed for 1 h at 37°C, after which it was terminated. The formation of 8-hydroxy tofacitinib was quantified by UPLC-MS/MS. The remaining enzymatic activities corresponding to each antidiabetic drug were ranked to assess their effects on the metabolism of tofacitinib.

The Effect of Two Hypoglycemic Drugs on in vitro Probe Substrate Metabolism

The incubation system consisted of 0.3 mg/mL RLM, 0.1 M potassium phosphate buffer and various concentrations of tofacitinib. The concentration of tofacitinib and column concentrations of linagliptin and gliquidone were determined to analyze the IC_{50} value. To assess the inhibitory effects of gliquidone and linagliptin on the metabolism of tofacitinib, the concentrations of gliquidone (0.25, 0.5, 1 and 2 μ M) and linagliptin (0, 1, 2, 4 and 8 μ M) were compared to tofacitinib (10, 20, 40 and 80 μ M) and the inhibitory constant (Ki) was calculated. The mixtures were incubated at 37 °C for 5 min. NADPH was added to initiate a 30-min reaction process and acetonitrile was added to terminate the reaction. UPLC-MS /MS was performed on the supernatant after centrifuging L for 2 min and separating the supernatant for 5 min at 13000 rpm. The data was analyzed using the GraphPad Prism 8.0 software. The Lineweaver Burk mapping method was used to determine the inhibitory types of gliquidone and linagliptin on tofacitinib and calculate the Ki values.

Pharmacokinetic Study in vivo

A total of 18 healthy male SD rats (average weighing 220±10 g) were randomly assigned to three groups: Gliquidone (Group A), Linagliptin (Group B) and control (Group C), each with six animals. Several days before the experiment, water and food were forbidden and 10 mg/kg of tofacitinib was administered weekly. Groups A and B were administered 20 mg/kg of gliquidone and 5 mg/kg of linagliptin, respectively and group C received a lysate control. Blood samples were collected from the tail veins of the rats at 0.083, 0.25, 0.50, 1, 2, 3, 4, 6, 8 and 12 hr after tofacitinib administration. The plasma samples were immediately separated from whole blood by centrifugation at 4000 rpm for 10 min and frozen at −80°C until analysis. UPLC-MS/MS mass spectrometry conditions were performed in rats following drug administration according to well-established methodological guidelines. The pharmacokinetic parameters were finally performed using DAS software (version 3.0, Lishui People's Hospital, China).

Pharmacokinetic Analysis

To analyze the pharmacokinetic parameters of tofacitinib, gliquidone and linagliptin, standard methods were applied using non-sectoral analysis (DAS 3.0). F represents the distribution of plasma concentration over time, as measured by the total area under the curve (AUC), versus the AUC following intravenous administration. The plasma concentration-time curve was used to determine the C_{max} and maximum plasma concentration (T_{max}).[35]

Molecular Docking Process

The crystal structure of CYP3A4 was downloaded from the PDB database website (https://www.rcsb.org/) as structure PDB file 2j0d. After that, tofacitinib, gliquidone, and linagliptin were downloaded in SDF format from PubChem (https://pubchem.ncbi.nlm.nih.gov/) and converted to PDB files using OpenBabel 3.1.1.[36] Molecular docking was performed with Autodock Vina after dehydrogenation by Autodock Tools 1.5.7.[37,38] The grid box size was set to 60Å×60Å×60Å and the grid box spacing was set to 0.3753Å. Tofacitinib, gliquidone and linagliptin were used for the docking of CYP3A4.

Statistical Analysis

In this study, $P<0.05$ was considered statistically significant. To calculate the pharmacokinetic parameters such as T_{max}, The terminal elimination half-life (t1/2z), the clearance rate (CLz/F), $MRT_{(0-t)}$, $MRT_{(0-\infty)}$, C_{max}, $AUC_{(0-t)}$, $AUC_{(0-\infty)}$, etc., from tofacitinib in rats, DAS3.0 software was utilized to analyze the blood concentration data from UPLC-MS/MS. The pharmacokinetic parameters from 6 rats per group (n=6) in vivo experiment were analyzed using one-way analysis of variance (ANOVA) in SPSS 25.0 (version 25.0; SPSS Inc., Chicago, IL). The enzyme kinetic parameters (maximum reaction rate V_{max}, K_m value, IC_{50}) and enzyme kinetic curves were calculated and plotted using GraphPad Prism version 8.0 (GraphPad Software Inc., San Diego, CA, USA). All data were reported as mean ± standard deviation (SD).

Results

In vitro Microsomal Incubation of Tofacitinib

The rat liver microsomal metabolizing enzyme produced the tofacitinib enzyme kinetic curve, which was analytically processed using GraphPad 8.0 (Figure 1). The results indicated that the V_{max} of the tofacitinib enzyme kinetic parameter was 5.482 pmol/min/mg and the K_m was 37.33 μM. The concentration of tofacitinib solution was optimized at 40 μM in the SD rat liver microsomal incubation system, as substrate concentration was typically near the Km value.

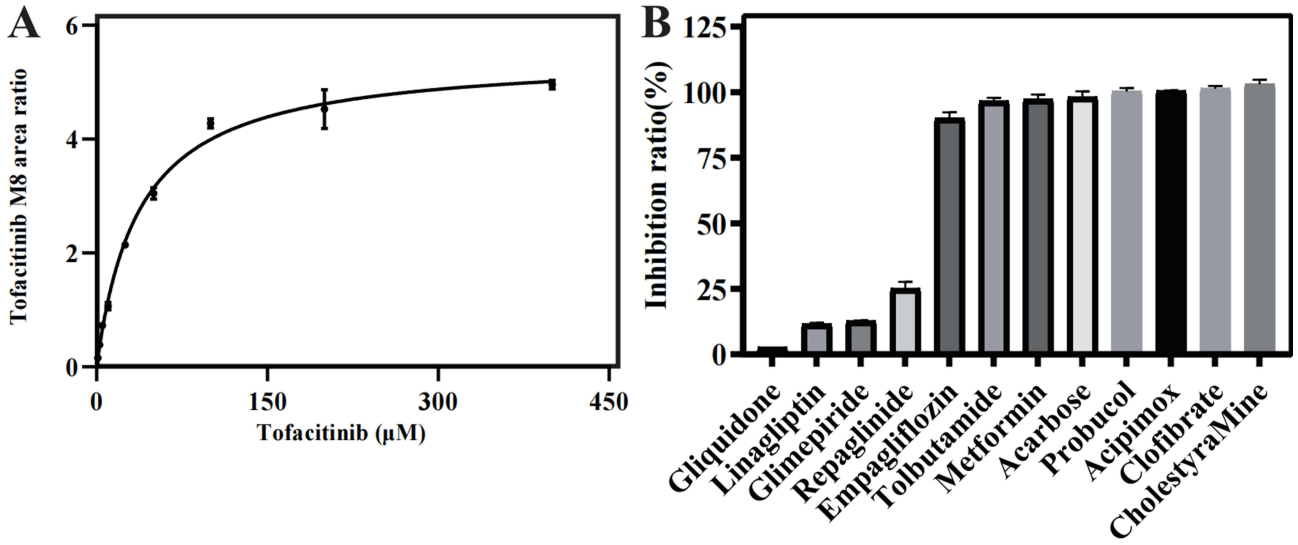

Figure 1 The Michaelis-Menten curve of tofacitinib metabolism to M8 (**A**), and the inhibition ratio of antidiabetic drugs on tofacitinib metabolism (**B**). (n=3).

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In vitro Microsomal Screening Inhibitors

Twelve glucose-lowering drugs (Gliquidone, Linagliptin, Glimepiride, Repaglinide, Empagliflozin, Tolbutamide, Metformin, Acarbose, Probucol, Acipimox, Clofibrate, and CholestyraMine) were added to tofacitinib in vitro during incubation. The results are shown in Figure 1B, in descending order: Gliquidone>Linagliptin>Glimepiride>Repaglinide>Empagliflozin>Tolbutamide>Metformin>Acarbose>Probucol>-Acipimox> Clofibrate>CholestyraMine. Tofacitinib metabolism was most robustly inhibited by gliquidone and linagliptin with residual activities ranging from 2.60% to 11.49% (Figure 1B), leading to the consideration of these agents as potential interactions.

The Inhibitory Effects of Tofacitinib by Gliquidone and Linagliptin in vitro

Three samples were analyzed for each concentration of gliquidone and linagliptin to inhibit tofacitinib in rat liver microsomes. The enzyme kinetics were fitted with the results of the measurements and the fitted curves are presented. The results showed that gliquidone and linagliptin inhibited tofacitinib by IC_{50} values of 1.140 and 4.064, respectively (Figure 2). In addition, Lineweaver-Burk plots confirmed that tofacitinib metabolism was inhibited by gliquidone from displaying a linear relationship between tofacitinib concentration and elimination rate. The effects of gliquidone (0, 0.25, 0.5, 1 and 2 μM) on tofacitinib were then investigated by co-incubating tofacitinib (10, 20, 40, and 80 μM) with rat liver microsomes. Figure 3 indicates that gliquidone tightly binds to the enzyme and inhibits tofacitinib in a mixed manner with anticompetitive inhibition being more pronounced. Tofacitinib (concentrations 10, 20, 40 and 80 μM) and linagliptin (0, 1, 2, 4 and 8 μM) were co-incubated with rat liver microsomes to investigate the various types of inhibition (Figure 4). In addition, Figure 4 shows that linagliptin inhibits tofacitinib with a Ki value of 3.144 μM and αKi value of 80.09 μM.

The Inhibitory Effects of Gliquidone and Linagliptin on Tofacitinib in Rats

The pharmacokinetic parameters for tofacitinib in three groups were determined (Table 1). Co-administration with gliquidone significantly altered the pharmacokinetic profile of tofacitinib. The $AUC_{(0-t)}$of tofacitinib was increased by approximately 43.3, reaching 3747.19 ± 993.86 μg/L*h, compared to the control group. Moreover, the C_{max} was elevated from 1189.38 ± 300.08 μg/L to 1725.63 ± 519.39 μg/L, representing a 45.1% increase. The clearance (CLz/F) was reduced by 27.5% from 3.85 ± 0.55 L/h/kg to 2.79 ± 0.58 L/h/kg. In contrast, linagliptin exhibited a much more potent inhibitory effect on tofacitinib's pharmacokinetics. The $AUC_{(0-t)}$ increased to 11565.17 ± 4363.28 μg/L*h, corresponding to approximately a 4.4-fold increase compared to the control group. Additionally, the C_{max} of tofacitinib was markedly

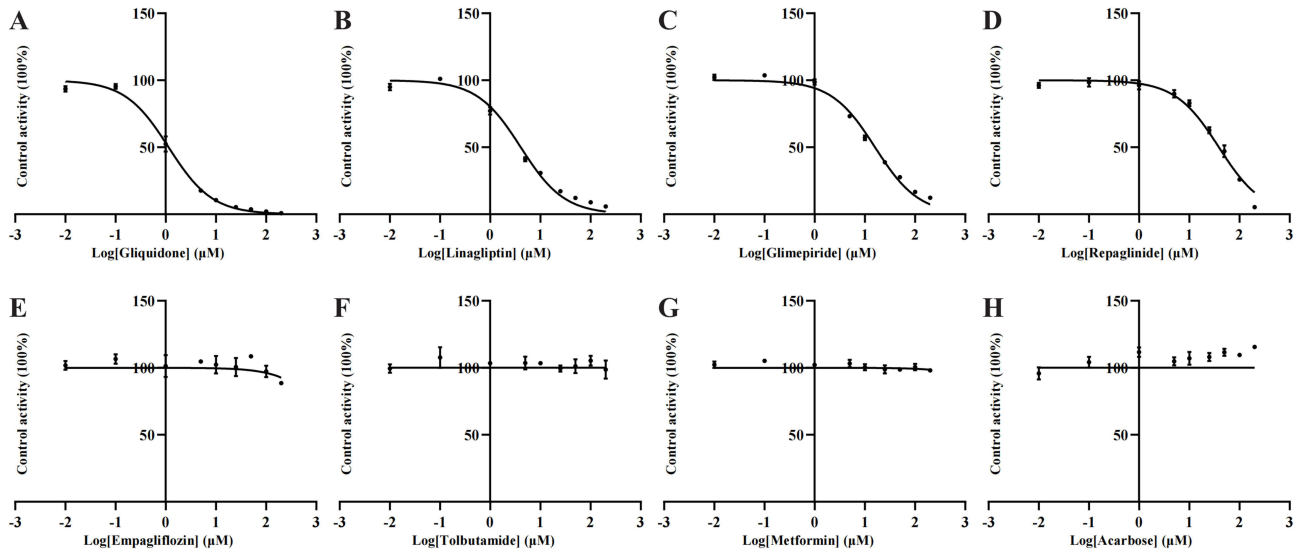

Figure 2 The inhibition IC_{50} value of antidiabetic drugs on tofacitinib. Gliquidone (**A**), Linagliptin (**B**), Glimepiride (**C**), Repaglinide (**D**), Empagliflozin (**E**), Tolbutamide (**F**), Metformin (**G**), and Acarbose (**H**). (n=3).

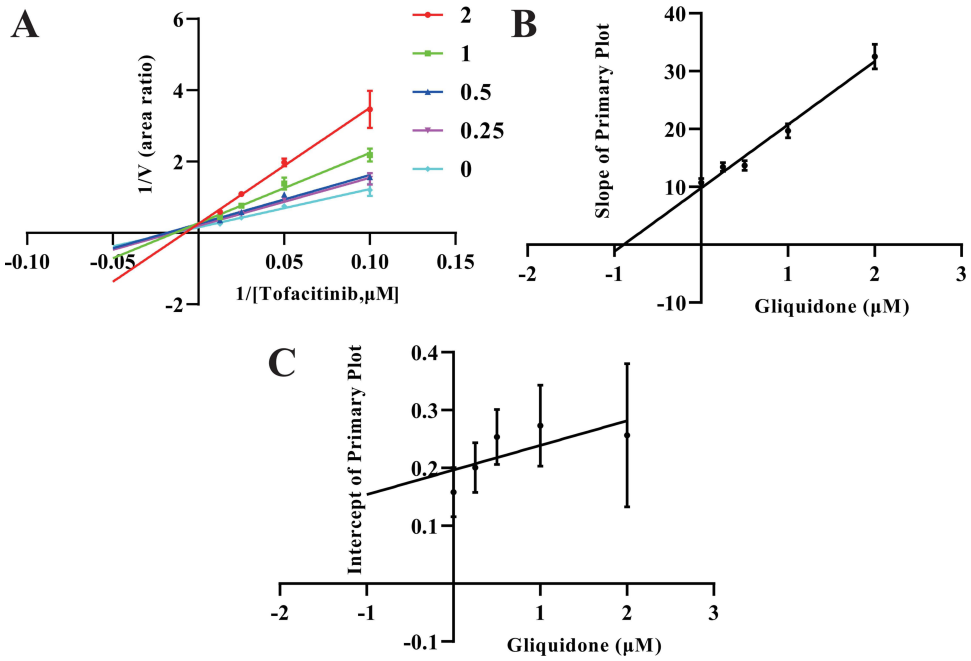

Figure 3 The inhibitory mechanism of gliquidone on tofacitinib metabolism. Lineweaver-Burk curve (**A**), slope of Lineweaver-Burk curve (**B**), and intercept of Lineweaver-Burk curve (**C**). (n=3).

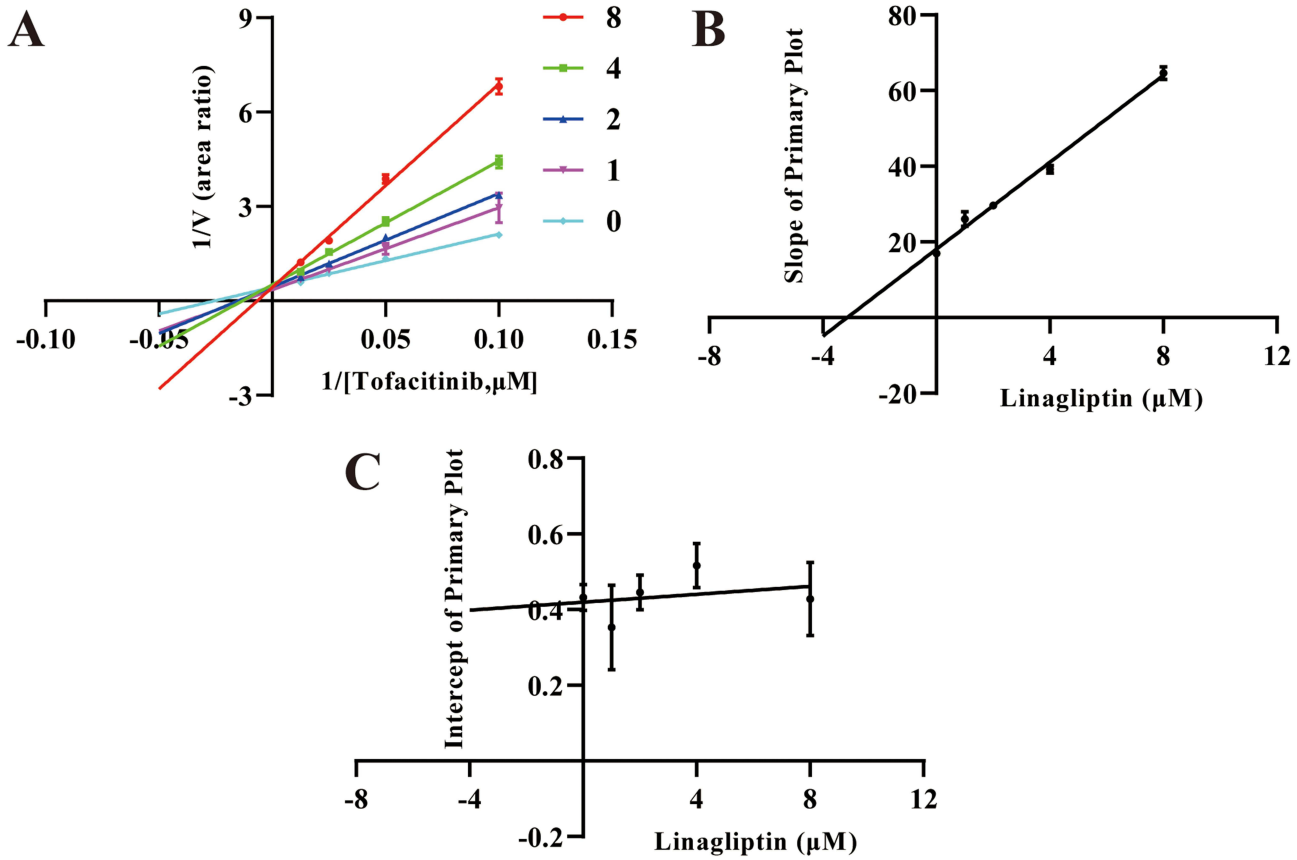

Figure 4 The inhibitory mechanism of linagliptin on tofacitinib metabolism. Lineweaver-Burk curve (**A**), slope of Lineweaver-Burk curve (**B**), and intercept of Lineweaver-Burk curve (**C**). (n=3).

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Table 1 The Primary Pharmacokinetic Parameters of Tofacitinib in Two Groups of Rats. (n = 6, Mean ± SD)

Parameters	Control Group	Gliquidone Group	Linagliptin Group
$AUC_{(0-t)}$ (μg/L*h)	2614.72 ±413.78	3747.19 ± 993.86*	11.565.17 ± 4363.28*
$AUC_{(0-\infty)}$ (μg/L*h)	2641.01 ±390.72	3757.72±991.464*	11.583.02±4361.32*
$MRT_{(0-t)}$ (h)	1.98 ± 0.36	1.72± 0.18	2.34± 0.22
$MRT_{(0-\infty)}$ (h)	2.13 ±0.56	1.75± 0.19	2.36±0.23
$t_{1/2z}$ (h)	2.03 ± 0.77	1.39± 0.57	1.27± 0.31
T_{max} (h)	0.29 ± 0.1	0.28 ±0.19	0.46 ±0.29
CL_z/F (L/h/kg)	3.85 ±0.55	2.79 ±0.58*	0.995 ±0.43*
C_{max} (μg/L)	1189.38 ±300.08	1725.63±519.39*	3400.22±1372.69*

Note: *Significantly different from the control group, $P < 0.05$.

elevated to 3400.22 ± 1372.69 μg/L, about 2.86 times higher than that of the control group. This significant enhancement in exposure was accompanied by a drastic reduction in clearance, decreasing to only 25.8% of the control level (from 3.85 ± 0.55 L/h/kg to 0.995 ± 0.43 L/h/kg (Figure 5). Taken together, both gliquidone and linagliptin significantly affected the pharmacokinetics of tofacitinib, with linagliptin demonstrating a remarkably more significant inhibitory effect than gliquidone.

Docking of Molecules

Molecular docking simulations were used to simulate the interaction between CYP3A4 and tofacitinib, gliquidone and linagliptin. As shown in Figure 6, CYP3A4 binds tightly to tofacitinib, gliquidone and linagliptin. The binding energies of tofacitinib, gliquidone and linagliptin to CYP3A4 proteins were −7.0, −9.2 and −9.0 kcal/mol, respectively. We found that tofacitinib binds to LEU-211 and PHE-304 of CYP3A4 through hydrogen bonding with distances of 2.5, 2.1 and 1.9 Å. In addition, we observed that gliquidone binds to GLU-374, GLU-374 and GLU-304 via hydrogen bonds with amino acid residues of GLU-374, GLU-374 and GLU-374 (Figure 6). The results also showed that linagliptin binds to amino acid residues ARG-105, GLU-374 and LEU-373 via hydrogen bonds at distances of approximately 3.1, 2.7 and 3.4 Å. Intriguingly, we found a highly overlapping spatial relationship between tofacitinib and the inhibitors gliquidone and linagliptin, which may explain the mixed inhibition of the CYP3A4 metabolism of tofacitinib by gliquidone and the competitive inhibition of tofacitinib by linagliptin.

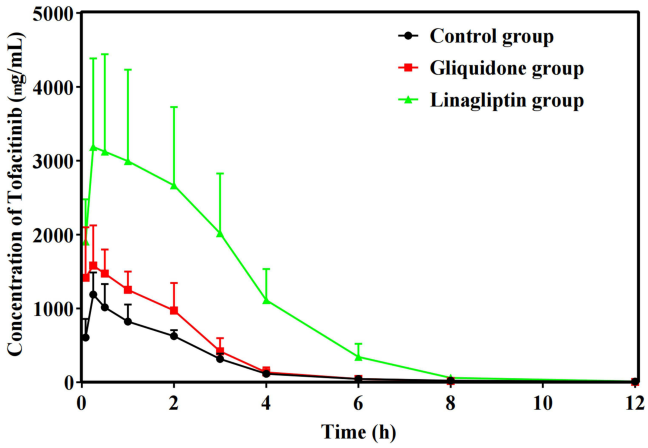

Figure 5 The concentration-time curves of tofacitinib after oral administration. (n=6).

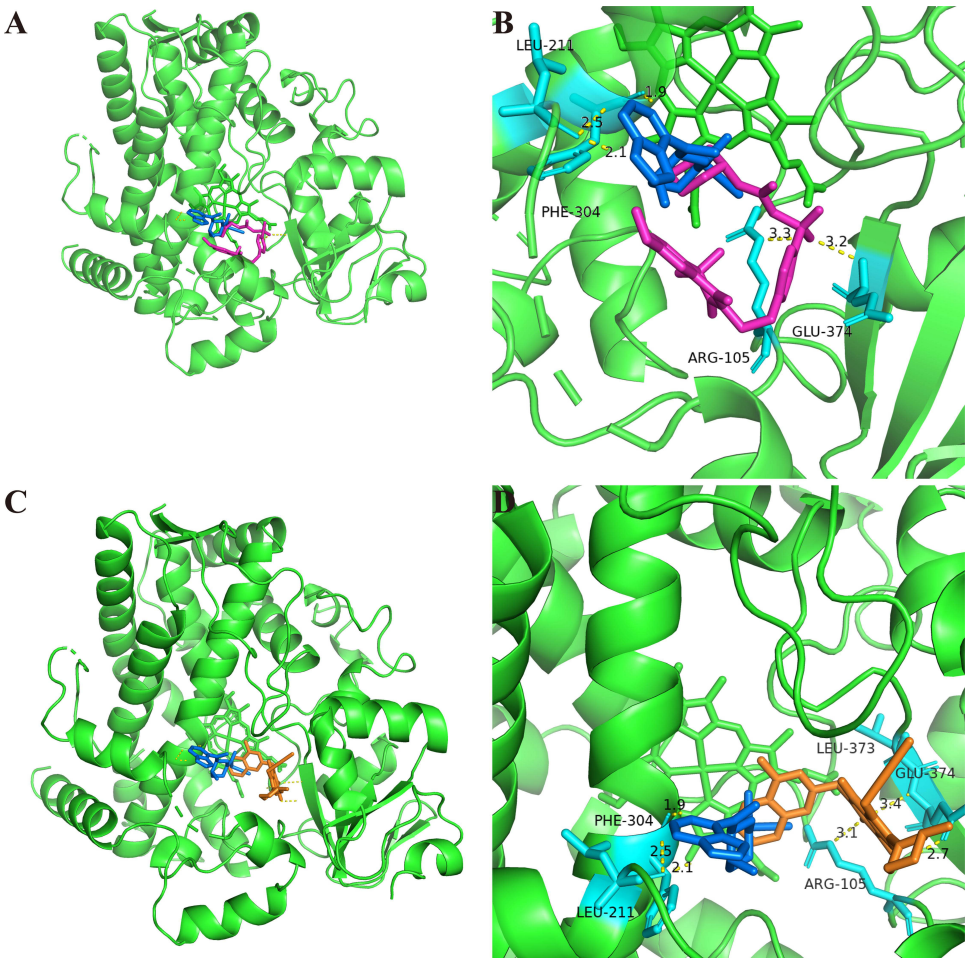

Figure 6 The docking simulations of antidiabetic drugs with the CYP3A4 protein (PDB ID: 2J0D). Overall view of CYP3A4 with gliquidone (**A**), detail view of CYP3A4 docked with gliquidone (**B**), overall view of CYP3A4 with linagliptin (**C**), and detail view of CYP3A4 docked with linagliptin (**D**).

Discussion

Tofacitinib and hypoglycemic drugs, such as gliquidone and linagliptin, are extremely common CYP3A4-mediated DDIs. However, these interactions are rarely discussed. T2DM comprises approximately 90–95% of all cases of DM and is associated with cardiovascular complications and mortality.[39] T2DM may be prevented and treated through lifestyle changes. However, pharmacological interventions are generally required. The effects of targeted anti-inflammatory therapy on RA may also reduce the symptoms of T2DM and its complications.[40] Tofacitinib can be beneficial in the management of chronic complications and the control of glucose levels.[6] However, tofacitinib may interact with hypoglycemic medications such as gliquidone and linagliptin, which may affect metabolism and efficacy. Therefore, the selection of a drug regimen should be made with caution. In this study, tofacitinib, linagliptin and gliquidone were systematically investigated for potential drug interactions.

In this study, we first screened twelve glucose-lowering drug inhibitors in vitro. The IC_{50} values of gliquidone and linagliptin on tofacitinib were 1.140 μM and 4.064 μM, respectively. We then evaluated the effects of gliquidone and linagliptin on the pharmacokinetics of tofacitinib both in vitro and in vivo. Two hypoglycemic agents, gliquidone and linagliptin, were then evaluated comprehensively on the pharmacokinetics of tofacitinib, both in vitro and in vivo, aiming to develop a safer and more effective pharmacological regimen for patients with RA and T2DM co-existing and provide them with appropriate guiding significance and reference values. We used the recommended daily dose of linagliptin 5 mg/kg according to the previously published work.[41] Thus, the study design is more likely to identify changes in PK parameters resulting from drug-drug interactions.

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It has been shown that tofacitinib and aspirin significantly improve insulin secretion and sensitivity by inhibiting JAK-STAT/NF-kB signaling pathways.[42] Therefore, tofacitinib and aspirin may be potential treatments for the management of T2DM. Additionally, several studies have examined the therapeutic use of JAK inhibitors in the treatment of RA. Patients with moderately to severely active RA who have not been adequately treated with other antirheumatic medications may benefit from taking tofacitinib, an FDA-approved drug. Research in this area has emerged as a new treatment option for RA.[5,43] Tofacitinib significantly downregulated the levels of TNF, IL-6, and serum amyloid A (SAA) in streptozotocin-induced diabetic rats.

Several studies have demonstrated that systemic exposure to tofacitinib increases significantly when combined with potent CYP3A4 inhibitors such as ketoconazole and fluconazole. Accumulating evidence suggests that the systemic exposure to tofacitinib decreases when combined with the potent CYP3A4 inducer levofloxacin.[6,10] Our results indicated that gliquidone inhibited the metabolism of tofacitinib through both competitive and non-competitive mechanisms, with a Ki value of 0.8937 μM and an αKi value of 4.617 μM. Moreover, we found that linagliptin inhibited tofacitinib competitively, with a Ki value of 3.144 μM. The data also indicated that the αKi value was 80.09 μM. Molecular docking studies were performed between gliquidone and linagliptin (ligands) and the human CYP3A4 enzyme (target), allowing a deeper understanding of amino acid residues, interaction energy and binding affinity. Molecular docking simulations revealed that tofacitinib and two hypoglycemic drugs, gliquidone and linagliptin, bind to CYP3A4 in highly overlapping spatial positions. Linagliptin is particularly susceptible to competitive inhibition by this overlap.

This study investigated the effects of gliquidone and linagliptin on the pharmacokinetics of tofacitinib in rats, revealing significant alterations in systemic exposure and clearance. Co-administration of gliquidone moderately increased tofacitinib exposure, as indicated by the approximately 43.3% and 42.3% increases in $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$, respectively, along with a 45.1% rise in C_{max} and a 27.7% reduction in clearance (CLz/F). The enhancement of tofacitinib exposure by gliquidone is likely due to the inhibition of metabolizing enzymes or transporters responsible for its clearance. Although the effect of gliquidone was significant, it was relatively mild compared to linagliptin. In contrast, co-administration of linagliptin resulted in a substantial increase in tofacitinib exposure, with $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ elevated by approximately 4.4-fold and C_{max} increased by 2.86-fold. This remarkable enhancement was accompanied by a dramatic reduction in clearance to only 25.8% of the control level. Such profound pharmacokinetic changes suggest that linagliptin may potently inhibit the metabolic enzymes and/or transporters involved in tofacitinib's elimination. Linagliptin, a DPP-4 inhibitor, has been shown to interact with substrates of CYP3A4, CYP2C9, CYP2C8, Pgp and organic cation transporters (OCTs) in vivo.[41] The pronounced difference in the effects of gliquidone and linagliptin on tofacitinib pharmacokinetics may be attributed to their varying affinities and inhibitory mechanisms toward enzymes and transporters. Notably, linagliptin exhibited a much stronger inhibitory effect, raising concerns about potential adverse effects when co-administered with tofacitinib. This finding highlights the importance of considering drug-drug interactions during combination therapy with tofacitinib, particularly with potent enzyme or transporter inhibitors such as linagliptin. Therefore, in clinical practice, when gliquidone and linagliptin glucose-lowering drugs are combined with tofacitinib, we recommend that clinicians consider adjusting the dose of tofacitinib or strengthening patient blood drug concentration monitoring.

There are also some limitations to our study. Our study demonstrates that linagliptin and gliquidone highly impaired the metabolism of tofacitinib. In contrast, the in vitro experiments presented in this study were conducted with a limited sample size and without statistical comparisons. These preliminary findings will be further validated in future studies with larger sample sizes and comprehensive statistical analyses.

The combination of gliquidone or linagliptin with tofacitinib may cause rational drug use. The results of this study were based on the analysis of rat liver microsomes and in vivo experiments conducted on rats. Further research on the results in humans is highly necessary. The outcomes of this study are still relevant for physicians when two medications are taken simultaneously in clinical settings and adverse drug responses or poor therapeutic effects occur.

Conclusion

This study provides valuable insights into the drug-drug interactions between tofacitinib and the antidiabetic agents gliquidone and linagliptin. Our findings demonstrate that both gliquidone and linagliptin significantly inhibit the hepatic

enzyme-mediated metabolism of tofacitinib, resulting in increased systemic exposure and reduced clearance of tofacitinib in rats. Notably, linagliptin exhibited a much stronger inhibitory effect compared to gliquidone. Molecular docking simulations further confirmed that both gliquidone and linagliptin bind to CYP3A4 in close spatial proximity, supporting the observed pharmacokinetic changes. These results emphasize the need for careful monitoring of potential drug interactions when co-administering tofacitinib with gliquidone or linagliptin in patients with T2DM and RA. Optimizing combination therapy through dosage adjustments or enhanced clinical surveillance could improve therapeutic safety and efficacy. Future studies are warranted to explore the clinical relevance of these interactions and to establish appropriate dosing guidelines for the safe co-administration of these agents.

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Disclosure

The authors report no conflicts of interest in this work.

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