

# Role of Glucuronidation Pathway in Quetiapine Metabolism: An *In vivo* Drug–Drug Interaction Study between Quetiapine and Probenecid

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## Abstract

**Background:** Uridine 5'-diphospho-glucuronosyltransferase (UGT) enzymes play a significant role in the metabolism of quetiapine, and coadministration with a UGT inhibitor/inducer drug may change its pharmacokinetic profile.

**Objective:** The objective of this study was to assess the impact of probenecid, a UGT enzyme inhibitor, on the pharmacokinetic profile of quetiapine.

**Materials and Methods:** Twelve treatment-naïve, 7-week-old male Sprague–Dawley rats (weighting  $161 \pm 22$  g) were randomly and equally divided into control, quetiapine-alone and quetiapine plus probenecid groups. The quetiapine plus probenecid group received a single oral dose of probenecid (50 mg/kg) followed by 50 mg/kg of quetiapine; the quetiapine-alone group only received 50 mg/kg of quetiapine. Blood samples (0.2 ml) were collected from all rats after 0, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h of the drug administration in heparinized tubes. The pre-established liquid chromatography–mass spectrometry method was utilized to ascertain the plasma concentration of quetiapine and the control group was used to prepare the controlled standard.

**Results:** Significant pharmacokinetic differences were observed between the quetiapine-alone and quetiapine plus probenecid groups in terms of  $C_{\max}$  ( $392 \pm 209$  vs.  $1323 \pm 343$  ug/L, respectively,  $P = 0.004$ ),  $AUC_{0-\infty}$  ( $P = 0.04$ ) and  $T_{\max}$  ( $P = 0.004$ ). Further, in the combined drug group, there was a decrease in drug clearance (CL/F) (from  $27 \pm 11$  to  $16 \pm 3$  L/h/kg;  $P = 0.005$ ) and an increase in the volume of distribution (Vd) ( $P = 0.01$ ), but there was no significant difference between both groups in terms of half-lives ( $P = 0.27$ ). No significant within-group variability of pharmacokinetic parameters was observed ( $P = 0.25$ ).

**Conclusion:** The results of this animal study suggest that glucuronidation by UGT enzyme system may also play an important role in quetiapine metabolism, which, if proven in future human studies, would imply that the bioavailability and pharmacokinetic parameters of quetiapine may require alterations when co-administered with probenecid to avoid development of quetiapine toxicity.

**Keywords:** Drug–drug interactions, liquid chromatography–tandem mass spectrometry, pharmacokinetics, probenecid, quetiapine, uridine-diphosphate inhibitor

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**Submitted:** 05-Oct-2019 **Revised:** 24-Dec-2019 **Accepted:** 04-Feb-2020 **Published:** 20-Aug-2020

Access this article online	
Quick Response Code:	Website: www.sjmms.net
	DOI: 10.4103/sjmms.sjmms_441_19

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**How to cite this article:** Sattar H, Jadoon SS, Yang N, Li S, Xu M, Han Y, *et al.* Role of glucuronidation pathway in quetiapine metabolism: An *in vivo* drug–drug interaction study between quetiapine and probenecid. Saudi J Med Med Sci 2020;8:196-200.

## INTRODUCTION

Drug metabolism is a pharmacokinetic pathway through which drugs are converted into a more water-soluble form to ease their elimination from the body. The liver is the main organ responsible for the metabolism and elimination of drugs. Some drugs are eliminated in bile as a conjugated form, while the water-soluble drugs are eliminated in urine. The most substantial conjugation reaction-related hepatic enzymes are uridine 5'-diphospho-glucuronosyltransferase (UGT) and cytochrome P450 (CYP). UGT enzymes are mainly present in the liver, but are also found in extrahepatic tissues, where glucuronidation converts drugs into conjugated form and helps eliminate them in feces.<sup>[1]</sup> UGT enzymes are known to carry out glucuronidation of some drugs such as ezetimibe, atorvastatin, ethinylestradiol, cerivastatin, gemfibrozil, ibuprofen, simvastatin, ketoprofen and buprenorphine.<sup>[2,3]</sup>

Quetiapine is a dibenzothiazepine-derived antipsychotic drug with a high *in vitro* binding ability for serotonin receptors 5-hydroxytryptamine-2. Quetiapine has a good absorption rate after oral intake and has been demonstrated to have a positive behavioral response in different animal prototypes with minimal extrapyramidal side effects.<sup>[4,5]</sup> This drug is mainly metabolized in the liver. The major biotransformation pathway comprises sulfoxidation by CYP 3A4 enzyme, which is mainly present in the gut and liver, and thus pharmacokinetic drug interactions may possibly occur when quetiapine is coadministered with CYP 3A4 inhibitors or inducers.<sup>[6]</sup> The hepatic UGT enzymes such as UGT 1A1, 2B7 and 2B7 are also located in the gastrointestinal tract and may influence the metabolism of drugs undergoing glucuronidation.<sup>[7]</sup> Numerous drugs have been documented *in vitro* as UGT enzyme inhibitors related to glucuronidation reactions, e.g., immune suppressants, anti-gout and anti-epileptics. These drugs can potentially cause serious adverse effects if the patient lacks UGT enzymes, especially in genetic disorders such as Gilbert syndrome and Crigler–Najjar syndrome.<sup>[8-10]</sup> Probenecid inhibits the various UGT isoenzymes such as UGT 1A1, 1A2, 1A3 and 2B7.<sup>[11]</sup>

For quetiapine metabolism, previous studies have mainly only focused on the CYP enzyme system, but whether the quetiapine metabolism also follows the glucuronidation pathway is still unclear.<sup>[7]</sup> Therefore, this study was conducted using male Sprague–Dawley rats with the objective of further validating the involvement of UGT enzymes and glucuronidation pathway in the metabolism of quetiapine and to confirm whether quetiapine metabolism can be altered when coadministered with a UGT enzyme inhibitor

such as probenecid. This rat model was chosen because it has been widely used to study the physiological-based *in vivo* pharmacokinetic drug–drug interactions, drug toxicology and UGT enzymes activity.<sup>[12,13]</sup>

## MATERIALS AND METHODS

### Ethical approval

Ethical approval for the animal experiments was obtained from the Ethics Committee of Huazhong University of Science and Technology on December 16, 2016. The animal experiments were carried out in accordance with the animal experiment guidelines of Huazhong University of Science and Technology.

### Chemicals

Quetiapine-d8-Hemifumarate (internal standard) was purchased from TLC Pharmaceuticals (Ontario, Canada), quetiapine fumarate and probenecid were purchased from Chemstrong Scientifics (Shenzhen, China), HPLC-grade methanol, acetonitrile, analytical-grade orthophosphoric acid and ammonium acetate were purchased from Sigma-Aldrich (Steinheim, Germany).

### Study design and experimental animals

This study was conducted between February 2017 and May 2017 and comprised three groups, namely, control group (no treatment), quetiapine group and quetiapine plus probenecid (QP) group. It was calculated that four rats in each group would provide a 80% power at a two-tailed  $\alpha$  level of 0.05 to detect the significant increase in quetiapine serum concentration in the QP group. Accordingly, 12 treatment-naïve, 7-week-old male Sprague–Dawley rats, weighting  $161 \pm 22$  g, were equally divided into three groups by simple randomization and numbered with a dye.

The rats were purchased 2 weeks before the start of the experiment and acclimatized. All rats were caged separately and fed at the Huazhong University of Science and Technology Tongji Medical College animal center with 12-h dark/light cycles.

### Experimental procedures

After a 15-h overnight fast, a single oral dose of probenecid (50 mg/kg; 0.6–0.8 ml of drug solution) was given to the QP group followed by 50 mg/kg of quetiapine (0.9–1.8 ml of drug solution) to both the quetiapine-only group and the QP group with the help of an oral gavage feeding needle; both drug solutions were given slowly to avoid any gastrointestinal complications. Blood samples (0.2 ml) from each group were collected from the tail vein with the help of insulin syringe after 0, 0.25, 0.5, 1, 2, 4, 6, 8, 10, 12 and 24 h in heparinized tubes containing 0.1 ml heparin.

Blood samples were centrifuged at 14,000 rpm for 5 min to collect blood plasma. Blood plasmas were then stored at  $-80^{\circ}\text{C}$  until further analysis.

### Plasma sample preparation for liquid chromatography–mass spectrometry

The control group was used to prepare the control standard for liquid chromatography mass–mass spectrometry (LC/MS). From each stored plasma sample, 50  $\mu\text{L}$  was transferred into a 2-ml Eppendorf tube and 30  $\mu\text{L}$  of the internal standard (100 ng/mL) and 1.5 mL of methanol were added. The samples were vortexed for 30 s and then centrifuged at 14,000 rpm for 3 min. From each tube, 5  $\mu\text{L}$  of the supernatant was collected for LC/MS injection.

### Liquid chromatography–mass spectrometry conditions

Positive ion MS conditions with two mobile phases consisting of 5 mm ammonium acetate (A) and acetonitrile (B) were used for the LC. Quetiapine-d8-Hemifumarate (260 ng/ml) was used as an internal standard. Quetiapine stock solution was prepared by dissolving 20 mg of quetiapine in 100 ml of methanol. From the stock solution, calibrating standard solutions were prepared with a range of 0.5–510 ng/ml. The Welch ultimate XB-C18 (20 mm  $\times$  50 mm, 5  $\mu\text{m}$ ) column (Welch Materials Inc. Shanghai, China) was used with a column temperature of  $35^{\circ}\text{C}$ . The dilutions of low-quality control (LQC), medium-quality control (MQC) and high-quality control (HQC) samples were 3 ng/ml, 100 ng/ml and 1600 ng/ml, respectively. The accuracy was determined by analyzing the LQC, MQC and HQC samples three times. The quantifications were obtained through multiple reaction monitoring of ion transition of quetiapine (384  $\rightarrow$  253 m/z) and internal standard (392  $\rightarrow$  258 m/z). The retention time of the internal standard was 1.04 min, and for quetiapine, it was 1.06 min.

### Outcomes measured

The primary endpoint of the research was to evaluate the significance of UGT enzymes for the metabolism of quetiapine and whether the inhibition of UGT enzymes can interfere with the quetiapine serum levels in rats. To avoid any bias in reporting, all assessments were made by one author who was not involved in the experiments and was blinded to the grouping.

### Data analysis

Unpaired *t*-test was used to observe significant differences between groups.  $P < 0.05$  was considered statistically significant. DAS 2.0 (Pharmacology Professional Committee of China, Shanghai, China) software was used to evaluate the pharmacokinetic parameters such as  $C_{\text{max}}$ ,

area under the curve (AUC) and plasma clearance (CL/F) of quetiapine alone and QP groups. GraphPad Prism V.6 (GraphPad Software, Inc., CA, USA) was used to calculate the *P* value;  $P < 0.05$  was considered statistically significant.

## RESULTS

The data of all animals included at the start of the study were available for analysis and no adverse event was noted in any rat from either group.

In the QP group, the plasma level of quetiapine was significantly increased as compared with the quetiapine alone group ( $155 \pm 37 \mu\text{g/L}$  vs.  $323 \pm 128 \mu\text{g/L}$ ;  $P = 0.05$ ). Similarly, the  $C_{\text{max}}$  of quetiapine also increased within an hour after drug administration in the QP group ( $392 \pm 209 \mu\text{g/L}$  vs.  $1323 \pm 343 \mu\text{g/L}$ ) ( $P = 0.004$ ). The decrease in the quetiapine clearance was significantly more in the combined drug group than in the quetiapine-alone group ( $27 \pm 11 \text{ L/h/kg}$  vs.  $16 \pm 3 \text{ L/h/kg}$ ;  $P = 0.01$ ). Interestingly, there was no significant difference noted in the half-life of quetiapine in both groups [Table 1]. The plasma concentration–time curves of the quetiapine and QP groups also indicate that probenecid alters the metabolism of quetiapine [Figure 1].

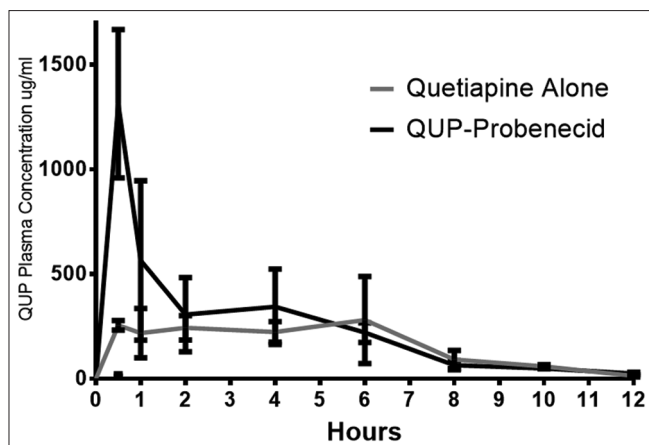
## DISCUSSION

Probenecid is a uricosuric drug that inhibits almost all types of glucuronidation-related enzymes and may interfere with the clinical pharmacokinetics of drugs undergoing glucuronidation.<sup>[14,15]</sup> In the current study, probenecid was found to significantly increase the  $C_{\text{max}}$  in the QP group as compared with the quetiapine-alone group, likely due to its inhibition of UGT enzymes. Notably, although there was an increase in the half-life of quetiapine in the QP group compared with the quetiapine alone group, this difference

**Table 1: Pharmacokinetic parameters**

Parameters	Mean $\pm$ SD		<i>P</i>
	Quetiapine alone	Quetiapine + Probenecid	
$C_{\text{max}}$ (ug/L)	392 $\pm$ 209	1323 $\pm$ 343	0.004*
$T_{\text{max}}$ (h)	5 $\pm$ 2	0.63 $\pm$ 0.3	0.005*
Vd/f (L/kg)	161 $\pm$ 115	209 $\pm$ 115	0.01*
AUC <sub>0-t</sub> (ug/L*h)	2778 $\pm$ 796	4067 $\pm$ 752	0.06
AUC <sub>0-<math>\infty</math></sub> (ug/L*h)	2817 $\pm$ 813	4313 $\pm$ 740	0.034*
$\lambda_z$ (h <sup>-1</sup> )	0.24 $\pm$ 0.2	0.134 $\pm$ 0.13	0.4
$T_{1/2}$ (h)	4 $\pm$ 2	9 $\pm$ 6.0	0.16
CL/f (L/h/kg)	27 $\pm$ 11	16 $\pm$ 3	0.001*
MRT (h)	4.5 $\pm$ 1.3	5.4 $\pm$ 2.5	0.5689

\*Statistically significant  $P < 0.05$ .  $C_{\text{max}}$  – Maximum plasma concentration;  $T_{\text{max}}$  – Time to maximum plasma concentration; AUC – Area under curve;  $\lambda_z$  – Elimination rate constant;  $T_{1/2}$  – Elimination half-life; CL – Clearance; MRT – Mean residence time; SD – Standard deviation



**Figure 1:** Plasma concentration–time curves of quetiapine and quetiapine plus probenecid

was not statistically significant, suggesting that quetiapine followed zero-order kinetics for elimination. The half-life of a drug is dependent on the volume of distribution (Vd) and its clearance (CL). The greater the CL, the lesser will be the half-life and vice versa, and the greater the Vd, the greater will be the half-life and vice versa. The reduced CL and high  $C_{max}$  of the quetiapine may suggest that these pharmacokinetic alterations may be due to the UGT enzyme inhibition by probenecid. Importantly, the reduced clearance rate could possibly lead to quetiapine toxicity.

Similarly to quetiapine and the findings of the current study, it has been found that probenecid also increases the level of olanzapine, which is an atypical antipsychotic drug metabolized by the CYP450 and UGT1A4 enzymes, by inhibition of UGT glucuronidation enzymes.<sup>[16]</sup> Probenecid is a nonselective inhibitor of UGT enzymes, and one of the reasons to select probenecid for this study was to confirm the quetiapine glucuronidation metabolism because it is yet unclear which UGT enzyme is responsible for this pathway.<sup>[14]</sup> In addition, there is no data available to confirm the *in vivo* probenecid and quetiapine drug–drug interactions. However, UGT inhibitors/inducers are known to alter the availability of quetiapine in the blood.

Valproic acid, a UGT enzyme and CYP450 inhibitor, has been found to increase the plasma level of quetiapine by 77% in elderly patients.<sup>[15]</sup> In fact, this interaction between valproic acid and quetiapine when used as a combination therapy for bipolar disorder can result in side effects such as leukopenia, thrombocytopenia and neutropenia, especially in the elderly population.<sup>[17,18]</sup> Valproic acid is a weak inhibitor of CYP3A4.<sup>[19]</sup> However, Santoro *et al.*<sup>[20]</sup> reported nonsignificant results when quetiapine was coadministered with valproic acid in adult patients. Similarly, when quetiapine was coadministered with

sodium divalproex in adult patients, its  $C_{max}$  was found to nonsignificantly increase by 17%, with no changes in AUC  $\tau$  in adult patients.<sup>[21]</sup> Taken together, these findings suggest that the quetiapine drug interactions are more significant in elderly patients due to the UGT enzyme inhibition. Our results are also similar with these finding; however, it needs to be further confirmed with probenecid in elderly patients.

In terms of UGT enzyme inducers, in one study that included 144 patients, administration of lamotrigine was found to decrease the plasma concentration of quetiapine by 17%,<sup>[22]</sup> while in another similar study with 22 patients, this decrease was by 58%.<sup>[23]</sup>

Although the current study found quetiapine and probenecid interaction in healthy rats, indicating that glucuronidation may influence quetiapine metabolism, there is a need for further validation of these findings using transgenic rats with knockout UGT enzymes gene and psychotic rat models to evaluate the pharmacokinetic profile and drug interaction in a diseased state. Consequently, there would also be a need to further validate these study findings in humans to establish these interactions.

## CONCLUSION

This study found significant *in vivo* drug–drug interaction between quetiapine and probenecid that resulted in a significant increase in the quetiapine plasma level, suggesting that glucuronidation may be another pathway by which quetiapine is metabolized. Such increase in quetiapine plasma level suggests that patients suffering from glucuronidation enzyme polymorphism disorders, such as Gilbert syndrome or Crigler Najjar syndrome, as well as gout patients using probenecid, could potentially be at risk of quetiapine toxicity; however, further studies are required to validate these findings.

## Ethical considerations

Ethics Committee of Huazhong University of Science and Technology, Wuhan, China, on December 16, 2016, and complied with the University's animal experiment guidelines.

## Peer review

This article was peer-reviewed by two independent and anonymous reviewers.

## Financial support and sponsorship

Nil.

## Conflicts of interest

There are no conflicts of interest.



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