Co-administration of Ritonavir and Primaquine Decreases Plasma Concentration of Primaquine: Single- and Multiple-dose Study in the Rats

Melva Louisa, Vivian Soetikno, Nafrialdi, Rianto Setiabudy, Frans D. Suyatna

Department of Pharmacology and Therapeutics, Faculty of Medicine, University of Indonesia. Jl. Salemba Raya no. 6, Jakarta Pusat 10430, Indonesia. Correspondence mail: melva.louisa@gmail.com.

ABSTRACT

**Aim:** to explore the effects of ritonavir and primaquine combination given as a single-dose or multiple-dose compared to ritonavir alone on ritonavir plasma concentration in the rats. **Methods:** in single-dose study, 30 male Sprague Dawley rats were randomly allocated to receive primaquine 12.5 mg/kgBW or primaquine 12.5 mg/kgBW + ritonavir 10 mg/kgBW or primaquine 12.5 mg/kgBW + ketokonazol 10 mg/kgBW. Ketokonazol was used as positive control for inhibitor of primaquine metabolism. In the multiple-dose study, thirty Sprague Dawley male rats were randomly allocated to receive primaquine 12.5 mg/kgBW/day or primaquine 12.5 mg/kgBW/day + ritonavir 10 mg/kgBW/day or primaquine 12.5 mg/kgBW/day + rifampicin 100 mg/kgBW/day. Rifampicin was used as a positive control for inducer of primaquine metabolism. **Results:** in the single-dose study, ketokonazol increases the area under the plasma concentration (AUC) of primaquine (∆45.8%, p<0.000), while the ritonavir decreases the AUC of primaquine (∆64.6%, p<0.000). Multiple-dose study shows that both rifampicin and ritonavir decreases the AUC of primaquine by 60.2% (p<0.000) and 67.7% (p<0.000), respectively. **Conclusion:** concomitant administration of primaquine and ritonavir decreases the AUC of ritonavir. This effect could result in the insufficient concentration of primaquine as anti-relapse therapy in malaria caused by Plasmodium vivax, which might lead to treatment failure with primaquine.

**Key words:** primaquine, ritonavir, drug interaction, metabolism.
INTRODUCTION

Two of the most deadly diseases, HIV and malaria, have an overlapping geographical distribution in sub-Saharan Africa and also in East- and South-East-Asia (India, Thailand, Myanmar dan Indonesia).1-3 There is a high likelihood of individuals in the endemic area like Indonesia being infected with these diseases. It is often related to the poverty and suitable environment for the pathogens to spread. Over 90 million people in Indonesia live in the malaria endemic area. In HIV/AIDS patients, immunodeficiency can affect the immune response of the body towards malaria parasites,1 while Hoffman et al., 1999 showed that in HIV patients with acute malaria, there are tendency towards higher HIV viral load. HIV infection can be easily transmitted from a patient with a high viral load. Therefore, HIV – malaria co-infection can increase the risk of HIV transmission.4 Hence, intensive intervention in the prevention and treatment of malaria in HIV/AIDS patients in endemic area is an effective way to reduce to risk of morbidity in these patients.

Concomitant treatment with antimalaria and anti-HIV is a new challenge in the management of co-infection of malaria/HIV. Drug interaction is an important aspect to consider to reach a successful clinical cure. Anti-HIV regimen consists of 3 to 4 drugs. If a patient has to receive a prophylactic treatment for malaria, then the patient receives two or three additional drugs.5 Primaquine is one of the drugs used in the therapy of malaria caused by Plasmodium vivax. P. vivax is one of the parasites which caused human malaria in the subtropics. Primaquine is the only drug available to eliminate hypnozoites in P.vivax. Regimen of primaquine to eliminate hypnozoites in the liver range from 14-day course to eight-week regimen.6,7 The administration of primaquine in a relatively long period is unavoidable in the endemic areas.

Interaction between antimalarial drugs and antiretroviral drug therapy mostly involve protease inhibitors.5 Ritonavir, is a peptidomimetic HIV protease inhibitor that is active against HIV-1 and HIV-2. Despite its activity as anti HIV, ritonavir is mostly used as a pharmacokinetic enhancer (CYP3A4 inhibitor).8 In an in vitro study, it was shown that primaquine is a substrate of cytochrome P4503A4 (CYP3A4),9 while ritonavir is known as substrate and inhibitor of CYP3A4.10 Theoretically, there is a high likelihood of these two drugs to interact via cytochrome P450.

In our previous study that investigated the effect of primaquine to the concentrations of ritonavir in the rat plasma, we reported that concomitant administration of ritonavir and primaquine, single-dose or repeated-dose, decreased the area under the plasma concentration curve of ritonavir (>40% reduction). We suggested that this effect could result in the insufficient concentration of ritonavir as anti-HIV, which might lead to treatment failure with ritonavir.11 Therefore, further information whether ritonavir might alter the plasma concentration of primaquine will add a valuable information in the treatment of malaria and HIV.

The aim of the present study is to investigate the effect of ritonavir on the plasma concentration of primaquine, given in single- or repeated-dose administration.

METHODS

Experimental Design

The study protocol was approved by the Ethics Committee of the Faculty of Medicine, University of Indonesia. The study consisted of two parts. The first part was designed to investigate whether ritonavir has inhibitory effect on the metabolism of primaquine, given concomitantly as a single dose. Ketokonazole was used as a positive control for inhibitor of primaquine metabolism. Thirty male Spraque Dawley rats weighing ±300 g were randomly allocated into 3 groups of 10 rats to receive primaquine 12.5 mg/kgBW or primaquine 12.5 mg/kgBW + ritonavir 10 mg/kgBW or primaquine 12.5 mg/kgBW + ketokonazole 10 mg/kgBW.

The second part was carried out to investigate whether ritonavir has inducing effect on the metabolism of primaquine, given concomitantly as a single dose. Ketokonazole was used as a positive control for inhibitor of primaquine metabolism. Thirty male Spraque Dawley rats weighing ±300 g were randomly allocated into 3 groups of 10 rats to receive primaquine 12.5 mg/kgBW/day or primaquine 12.5 mg/kgBW + ritonavir 10 mg/kgBW or primaquine 12.5 mg/kgBW + rifampicin 100 mg/kgBW.
Blood Samples
For single-dose study, serial blood samples were collected at 0, 1, 2, 3, 4 and 6 h after drug administration. For repeated-dose, study serial blood samples were collected at day 0 (before drug administration), 1, 2, 3, 4 and 5. Blood samples were centrifuged at 3000 g for 15 minutes at 40°C and the separated serum was assayed on the same day.

Analytical Methods

HPLC methods to determine primaquine in plasma. The concentration of primaquine in plasma was analysed by a validated HPLC method with ultaviolet detector at $\lambda=255$ nm. The mobile phase was KH2PO4 buffer pH 5.8 : methanol : acetonitrile = 65 : 15 : 20, pumped isocratically at flow rate of 1.0 mL/min and temperature of 350°C. We use phenacetine as internal standard. Retention time of phenacetine and primaquine were at 5.3 and 6.3 min, respectively (Figure 1). Ritonavir could not be detected in this system.

Sample extraction. Plasma sample (0.25 mL) was spiked with 50 µL phenacetine (internal standard) and 50 µL NaOH 0.1 N. The mixture was vortexed for 30 seconds before adding 1.5 mL of dichlormethane. Then, the sample was centrifuged at 3000 rpm for 5 minutes. The organic layer was evaporated under a gentle stream of nitrogen. The residue was reconstituted in 200 µL of mobile phase and an aliquot of 20 µL was injected to the HPLC system (WatersTM). The mixture was separated on a Reverse Phase C18 Column (SymmetryTM C18 5 µm; 2.6 x 150 mm).

Analytical system validation. The calibration curve was linear ($r = 0.9999$, $n = 6$) in the range of 0.5 – 6 µg/mL, and the limit of quantitation was 0.9 µg/mL. Precision, expressed as the inter- ($n=5$) and intraday ($n=10$) coefficient of variation, was $\leq 7.84\%$ on the same day and $\leq 10.45\%$ between days for each quality control sample of 1.5; 2.5 and 5 µg/mL, respectively. Accuracy expressed as the inter- and intraday% bias was 0.04–1.83% and -2.88–1.56% between days at each quality control sample.

Statistical Analysis
AUC of ritonavir was calculated using the trapezoidal rule based on plasma concentrations obtained at the predetermined sampling time points as stated in methods.

Since the data obtained showed a normal and homogeneous distribution, AUC0-t(h) of primaquine concentration were analyzed using the related one-way ANOVA followed by Tukey method for multiple comparisons at a significance level of $\alpha = 0.05$. Results are expressed as means ± S.D.

RESULTS
The mean concentration of primaquine in a single dose study and repeated dose study versus
time was shown in Figure 2.

In the single dose study, concomitant administration of primaquine with ketokonazole showed an increase in the area under the plasma concentration (AUC) of primaquine compared to primaquine alone (increased by 45.8%, p<0.000). This result was as expected before. Ketokonazole is a potent inhibitor of CYP3A4, an enzyme which also metabolizes primaquine. Inhibition of metabolizing enzyme caused a significant increase in the plasma concentration of primaquine. On the contrary, concomitant administration of primaquine + ritonavir significantly lowered the AUC of primaquine (decreased by 64.6%, p<0.000) compared to primaquine alone (Figure 3A).

The result of the repeated dose study, concomitant administration of primaquine with rifampicin showed a decrease in the AUC of primaquine (decreased by 60.2%, p<0.000). This result was also as expected. Rifampicin is the known inducer of cytochrome P450, not only the CYP3A4 but also several other cytochrome P450 isozymes. It was known from previous publications that the induction of cytochrome P450 in the rats might occur 3–5 days after drug exposure. Concomitant administration of primaquine with ritonavir given repeatedly for 5 days also significantly lowered the AUC of primaquine (decreased by 67.7%, p<0.001) (Figure 3B).

Figure 2. Mean of ritonavir concentration versus blood sampling time after. A. Single dose administration of primaquine 12.5 mg/kgBW or primaquine 12.5 mg/kgBW + ritonavir 10 mg/kgBW or primaquine 12.5 mg/kgBW + ketokonazole 10 mg/kgBW; B. Repeated dose administration for 5-days of primaquine 12.5 mg/kgBW/day or primaquine 12.5 mg/kgBW/day + ritonavir 10 mg/kgBW/day or primaquine 12.5 mg/kgBW/day + rifampicin 100 mg/kgBW/day

Figure 3. Mean Area Under the Curve (AUC) of primaquine concentration after administration of: A. single dose of primaquine, primaquine + ritonavir, primaquine + ketokonazole. B. repeated administration of primaquine, primaquine + ritonavir, primaquine + rifampicin
DISCUSSION

The present study shows that single concomitant administration of ritonavir and ketoconazole results in the significant increase of primaquine plasma concentration, while ritonavir significantly reduces plasma concentration of primaquine.

Interaction between primaquine and ketoconazole was as expected before. Ketoconazole increases AUC of primaquine by 45.8%. This significant increase could lead to the higher toxicity of primaquine. Higher doses of primaquine is known to cause many undesirable effects, such as mild to moderate cramps and occasional epigastric distress. These symptoms are often accompanied by methemoglobinemia and cyanosis. Ketokonazole increases AUC of primaquine by 45.8%. This significant increase could lead to the higher toxicity of primaquine. Higher doses of primaquine is known to cause many undesirable effects, such as mild to moderate cramps and occasional epigastric distress. These symptoms are often accompanied by methemoglobinemia and cyanosis. Ketokonazole increases plasma concentration of primaquine through the inhibitory mechanism to cytochrome P450, mainly the CYP3A4 isoform. Primaquine is a substrate of CYP3A4 and CYP1A2, while ketoconazole is a potent inhibitor of CYP3A4.9,15 The capacity of ketoconazole to impair biotransformation of CYP3A is well established in in vivo and in vitro models.15,16 Though ketoconazole is commonly utilized as an index inhibitor of human CYP3A4 isoforms, the mechanism of ketoconazole inhibition of CYP3A is still not clearly established.16,17 Greenblat et al., showed that the mechanism of ketoconazole inhibition to CYP3A4 appeared to be a mixed competitive-noncompetitive process, with the noncompetitive component being dominant but not exclusive.17 Another research by Lim et al., showed that inhibition of CYP3A4 by ketoconazole can also be mediated through the disruption of pregnane X receptor, steroid receptor coactivator-1 and hepatocyte nuclear factor 4 [alpha] interaction.18

No direct interaction study between primaquine and ketoconazole has been done before, in animals or in human. Therefore, we could not compare the magnitude of the decrease in primaquine AUC in our result with those in other studies.

Single concomitant administration of ritonavir and primaquine reduced area under the plasma concentration of primaquine by 64.6%. Ritonavir was known for its inhibitory properties to CYP3A4.

A study by von Moltke showed that ritonavir are a highly potent mechanism-based inhibitors of human CYP3A isoforms. In their study, von Moltke et al., showed that ritonavir and amprenavir inhibitory properties were as potent as ketoconazole.16 However, in our result, ritonavir did not act as inhibitor of primaquine metabolism. On the contrary, ritonavir even reduced the AUC of primaquine. The possible explanation of this reduction might be through the interaction in the absorption site. Our previous study also showed that concomitant administration of ritonavir and primaquine also resulted in the reduction of ritonavir plasma concentrations.

Our result showed that repeated-dose concomitant administration of primaquine + rifampicin or primaquine + ritonavir, both significantly decreased the AUC of primaquine. The significant decrease of primaquine plasma concentrations could result to the insufficient concentration of primaquine as anti-relapse in the treatment of malaria caused by P.vivax.

It is widely known that rifampicin is an inducer of cytochrome P450. Rifampicin potently induces CYP1A2, CYP2C9 and CYP3A4. Its administration in a standard dose often results in a reduced plasma concentrations and altered drug efficacy of a large number of compounds, including antimalarials such as quinine, chloroquine, mefloquine and artemisinins. However, no previous data has ever described the influence of rifampicin to the pharmacokinetics and pharmacodynamics of primaquine.21

Our results showed that both single- and repeated-dose concomitant administration of ritonavir and primaquine reduced the AUC of primaquine. The decrease is significant, which lowered of primaquine AUC of 67.7% (in repeated-dose study), which is somewhat greater reduction than that caused by rifampicin (60.2%). This decrease could result to the insufficient concentration of primaquine as anti-relapse in the treatment of malaria caused by P.vivax, which might lead to treatment failure and drug
resistance with primaquine. We suggests that the reduction of primaquine plasma concentrations was due to the inducing effect of ritonavir to metabolizing enzyme, CYP3A4. Despite the inhibitory properties of ritonavir to CYP3A4, ritonavir was also known as moderate inducer of CYP3A4. Foisy in 2008, showed that at both therapeutic and boosting doses, ritonavir exhibits a clinically relevant induction effect on numerous drug metabolizing enzymes. A decrease or loss of therapeutic effect may be observed when ritonavir is coadministered with medications that are substrates for these enzymes.22

Ritonavir is a unique drug that exhibits both inhibitory and inducing effects on metabolizing enzymes. Therefore, clinicians should be aware of the potential of ritonavir in altering plasma concentrations of many drugs that shared the same metabolizing enzymes. Primaquine is one of the drugs that was impacted by ritonavir. Clinicians should be aware of this interactions. Since primaquine is the only drug available to prevent relapses in malaria caused by P.vivax, a decrease efficacy of primaquine due to concomitant administration with ritonavir is a serious problem to consider.

However, our study was done in animal model. This model was limited by species differences in systemic pharmacokinetics.13 Our result still needs to be confirmed in clinical trials that investigated full pharmacokinetic parameters of primaquine.

CONCLUSION
As a conclusion, this study showed that single- or repeated-dose concomitant administration of ritonavir and primaquine decreased plasma concentration of primaquine in animal models. This effect could result in insufficient concentration of primaquine as anti-relapse therapy in malaria caused by Plasmodium vivax, which might lead to treatment failure with primaquine.

REFERENCES