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# Effect of Ticagrelor Variable Doses on the Pharmacokinetic Parameters of Tacrolimus in Rabbits

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## Abstract:

*Tacrolimus (TAC) is a potent immunosuppressant drug which is used as a prophylaxis treatment mainly in kidney transplantation to prevent organ rejection. TAC is predominantly metabolized by CYP3A4 and it is also likely to be a substrate for the P-glycoprotein (P-gp) efflux pump. Ticagrelor (TICA) is a new modified antiplatelet drug that has been shown to be a weak inhibitor for both CYP3A4 isoenzyme and P-gp. The purpose of this study is to evaluate the effect of TICA at various doses on TAC pharmacokinetic (PK) parameters in rabbits. An in vivo parallel-randomized drug-drug interaction study was conducted using fifteen healthy male rabbits divided into three groups (n=5 rabbits for each). In the control group, TAC (0.6 mg/kg/day) was administered as a single daily oral dose for six consecutive days, while in the test groups, TAC (0.6 mg/kg/day) was concomitantly co-administered with TICA at concentrations of 10 or 20 mg/kg/day. On the sixth day of administration, serial blood samples (2-3 ml) were obtained from the rabbits' ear marginal veins at the following time points (0.0, 15.0, 30.0, 45.0, 60.0, 120.0, 180.0, 300.0 and 420.0 minutes) from the three groups. TAC concentrations in the blood were measured by using the*

*chemiluminescent enzyme immunoassay (CLEIA) procedure, and the PK parameters of TAC in the three groups were calculated using non-compartmental analysis. Our findings revealed statistically insignificant differences in the mean of the PK parameters between three groups. In conclusion, it has been found that TICA at both concentrations does not affect the TAC PK. Further confirmation of our findings is required in humans before these results can be applied in patient care.*

**Keywords:** Drug-drug interaction, pharmacokinetic parameters, tacrolimus, ticagrelor, CYP3A4.

## Introduction

Drug-drug interactions (DDI) can bring about a change in efficacy or lead to toxicity in one or both of the interacting agents (Becker *et al.*, 2005). TAC is a macrolide immunosuppressive drug used to prevent organ rejection in patients whose transplanted organs or tissues (Velickovic *et al.,* 2015). TAC has a narrow therapeutic index, as well as inter- and intra-individual variability (Christians *et al.,* 2002). To minimize the fluctuation in the immunosuppressive activity and the potential for drug interactions, it's essential to keep track of drug concentrations (Van *et al.,* 2002). Consequently, all transplanted patients who use TAC as a prophylactic agent against organ rejection should perform regular measurements of TAC concentrations in their blood. TAC has a low bioavailability (about 20%) due to its extensive pre-systemic metabolism (Iwasaki *et al.,* 2007; Wallemacq *et al.,* 2001). TAC is a substrate of the P-gp efflux pump, which is one of the drug transporters that controls its uptake and efflux (Finch *et al.,* 2014). Because TAC has a narrow therapeutic index, it's essential to quantify its blood level accurately and assess the impact of any interfering drugs (Gantar *et al.,* 2020). In addition, TAC is a substrate of CYP3A4 and CYP3A5, where it is primarily metabolized through the CYP3A4 enzyme (Amundsen *et al.,* 2012). TAC is highly lipophilic and is excreted from the body after receiving intensive metabolism (Iwasaki *et al.,* 2007). Ticagrelor (TICA) is a new short acting antiplatelet agent related to a chemical group known as

cyclopentyl-triazole-pyrimidines (CPTP) (Husted *et al.,* 2006). TICA is extensively metabolized by CYP3A4 and CYP3A5 (VAN *et al.,* 2009). In healthy subjects, TICA is rapidly absorbed, with a median time to peak concentration ($t_{max}$) of 1.3-2 hours, and after absorption, TICA is highly bound to plasma proteins (Teng 2015). TICA has been shown in vitro to be a weak inhibitor and activator of CYP3A4, as well as a substrate and weak inhibitor of P-gp (Zhou *et al.,* 2011). As a consequence, co-administration of drugs that inhibit or induce CYP3A4 such as TICA may alter TAC metabolism and may result in the development of TAC toxicity or diminish its efficacy, which may increase the probability of organ transplant rejection (Christians *et al.,* 2002; Rancic *et al.,* 2015). The present study was carried out to investigate the effect of co-administration of TICA at different doses on the PK profile of TAC in healthy male rabbits.

## Materials and Methods

### Chemicals

TAC capsules (5 mg) PROGRAF® *(FK 506)* kindly gifted from drug stores by the Palestinian Ministry of Health. The capsule content was dispersed in distilled water prior to administration. TICA tablet (90 mg) BRILINTA® was purchased from a local pharmacy (Gaza, Palestine). The tablet was dispersed in water immediately then given to the rabbits by a special oral gavage.

### Animals and study design

In *an in vivo* randomized, parallel controlled designed study, fifteen adult male rabbits of the New-Zealand strain (weighted 3.1-3.4 kg and aged 8-10 months) were enrolled in a drug-drug interaction study between TICA and TAC. The study was approved by the Experimental Animal Care Center, Faculty of Pharmacy, Al-Azhar University, Gaza, Palestine. The rabbits were maintained under the regulations of the "Guide for the Care and Use of Laboratory

Animals." The rabbits were maintained under standard laboratory conditions of a 12-hour light/dark cycle at 25°C ± 2°C and given a pellet diet with free access to water (*ad libitum*) and fasted overnight prior to the experiments.

The rabbits were divided into three groups (each with five rabbits) as follows: TAC (0.6 mg/kg/day) was administered alone as a single daily oral dose for six days in the control group, whereas, in the first and second test groups TAC (0.6 mg/kg/day) was co-administered with (10 and 20 mg/kg/day) TICA respectively.

## Blood sampling and analysis

Serial venous blood samples (2-3 ml) were obtained from rabbits' ear marginal veins for analysis on the sixth day of drugs administration at the following time points (0.0, 15.0, 30.0, 45.0, 60.0, 120.0, 180.0, 300.0, and 420.0 minutes) from the three groups. The method of determining the concentration of TAC in the blood is based on the measurement of TAC blood concentrations using an ARCHITECT-analyzer 1000 (Abbott Laboratories, Abbott Park, IL, USA) by a chemiluminescent enzyme immunoassay (CLEIA) bioanalytical procedure. All procedures, calibration standards, and validation followed the supplier's working protocols. The lower detection limit was 0.3 ng/mL, and the assay was calibrated from 2.4 to 30.0 ng/mL (Egashira *et al.,* 2012).

## Determination of PK parameters and statistical analysis

PK parameters for control and test groups, including: $C_{max}$, $t_{max}$, $K_e$, $AUC_{0\text{-}420}$, and MRT, were determined for three groups. Both parameters ($C_{max}$) and ($t_{max}$) were directly determined from the plasma concentration versus time curves. The $AUC_{0\text{-}420}$ was calculated by the linear trapezoidal rule. The $K_e$ was determined by the least squares regression of plasma concentration-time data points lying in the terminal region using semilogarithmic dependence that corresponds to first-order kinetics. PK analysis was determined by means of the

model independent method (Non-Compartmental Approach), WinNonlin Professional Software (Version 6.3, Pharsight Corporation, Cary, NC) and (GraphPad Prism version 4.00; San Diego, CA, USA). The Kruskal-Wallis test is a non-parametric test which is used to compare the distribution across the three groups. (SPSS) program (version 22.0) was applied to analyze the data. A statistically significant difference was considered when $P \leq 0.05$.

## Results and Discussion

This study was carried out to determine the influence of TICA at two different concentrations (10 and 20 mg/kg/day) on PK parameters of TAC (0.6 mg/kg/day) when given concurrently. The PK parameters of TAC in the control group were compared with those of the test groups treated with TICA 10 and 20 mg/kg/day (first and second test groups respectively). Figure 1 shows the plot of mean blood concentrations versus time curve profile of TAC in control, first and second test groups.

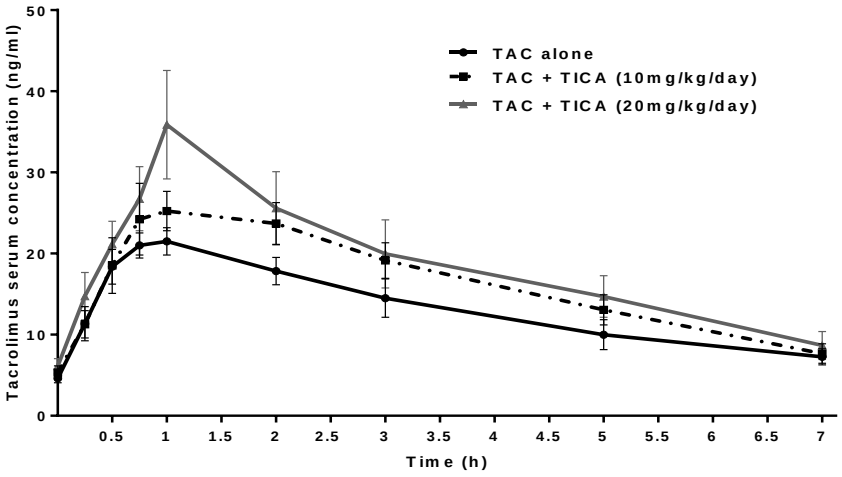


**Figure 1: Effect of TICA coadministration on tacrolimus concentrations.**

The calculated PK parameters: $C_{max}$, $t_{max}$, $K_e$, $AUC_{0\text{-}420}$ and MRT for the three groups were mentioned in table 1. In this study, $C_{max}$ of TAC

(control group) was 23.6 ± 7.88 ng/mL and $t_{max}$ was 1.0 ± 0.0 h. The rabbits treated with TICA 10 mg/kg/day (first test group) produced $C_{max}$ and $t_{max}$ of 32.57 ± 10.65 ng/mL and 1.0 ± 0.75 h, respectively. The increase in $C_{max}$ of TAC when TICA was co-administered (first test group) with TAC was statistically insignificant. Other PK parameters, including $K_e$, $AUC_{0\text{-}420}$ and MRT, showed no significant differences between both groups. Also, the estimated PK parameters of TAC alone (control group) and when co-administered with TICA 20 mg/kg/day (second test group) were also listed in table 1. In this case, the differences in $C_{max}$, $t_{max}$, $k_e$, $AUC_{0\text{-}420,}$ and MRT of TAC were statistically insignificant $P \geq 0.05$.

**Table 1:** Calculated PK parameters of TAC in control, first and second test groups.

| PK Parameters | Groups | N | Mean ± SD | Median ± IQR | P-Value |
|---|---|---|---|---|---|
| [a]$C_{max}$ (ng/mL) | Control group | 5 | 23.60 ± 7.88 | 21.70 ± 8.37 | 0.174 |
| | First test group | 5 | 32.57 ± 10.65 | 28.94 ±12.15 | |
| | Second test group | 5 | 35.89 ± 28.48 | 40.76 ± 25.33 | |
| [b]$T_{max}$ (hr) | Control group | 5 | 1.00 ± 0.00 | 1.0 ± 0.32 | 0.513 |
| | First test group | 5 | 1.00 ± 0.75 | 1.0 ± 0.50 | |
| | Second test group | 5 | 1.00 ± 0.00 | 1.0 ± 0.00 | |
| [c]$k_e$ ($hr^{-1}$) | Control group | 5 | 0.122 ± 0.096 | 0.0975 ± 0.10 | 0.775 |
| | First test group | 5 | 0.078 ± 0.081 | 0.087 ± 0.075 | |

| | | | | | |
|---|---|---|---|---|---|
| | Second test group | 5 | 0.073 ± 0.098 | 0.089 ± 0.108 | |
| [d]$AUC_{0-420}$ (ng*hr/mL) | Control group | 5 | 146.49 ±78.16 | 149.3 ± 75.1 | 0.468 |
| | First test group | 5 | 171.91± 41.99 | 169.2 ± 31.16 | |
| | Second test group | 5 | 152.06 ± 66.5 | 156.0 ± 43.6 | |
| [e]MRT | Control group | 5 | 2.74 ± 0.41 | 2.805 ± 0.37 | 0.494 |
| | First test group | 5 | 2.82 ± 0.22 | 2.751 ± 0.21 | |
| | Second test group | 5 | 2.86 ± 0.06 | 2.82 ± 0.21 | |

*P≤0.05 Statistical significance, SD: Standard deviation, [a]maximum blood concentration, [b]time of peak concentration, [c]elimination rate constant, [d]area under the concentration-time profile curve from 0 to 420 minuts and [e]Minimum residence time.

TAC is widely used to prevent allograft rejection in patients with transplanted organs or tissues (Bowman and Brennan 2008). So, it is more investigated in drug research, focusing on the transplantation area. TAC has a large inter- and intra-individual PK variability with a narrow therapeutic range (Gantar *et al.,* 2020; Rancic *et al.,* 2015). Because of the role of CYP3A and the P-gp efflux pump system in TAC metabolism, many pharmacological interactions have been described between TAC and other drugs (Keven *et al.,* 2004). In general, close monitoring of TAC blood levels is mandatory when combined with other drugs affecting its metabolism in long-term use. TICA, a new antiplatelet agent, is metabolized by CYP3A4 and CYP3A5 (VAN *et al.,* 2009; Amundsen *et al.,* 2012). It has been shown that TICA is a weak inhibitor and activator of CYP3A4, as

well as a substrate and weak inhibitor of P-gp (Zhou *et al.,* 2011). Consequently, co-administration of TICA and TAC may affect the PK profile of TAC. The current study was conducted to assess the potential drug-drug interactions between these two drugs when administered concomitantly.

Drug interactions with TAC due to affecting the activity of CYP3A enzymes and P-gp could affect its blood concentrations. Likewise, drugs inhibiting CYP3A enzymes may cause significant increases in TAC blood concentrations, causing critical TAC toxicity, while inducers of CYP3A enzymes may reduce their blood concentrations, which may lead to therapeutic failure and organ rejection (Rancic *et al.,* 2015; Vavić *et al.,* 2016; Yu *et al.,* 2018).

Our results showed that co-administration of TICA at different concentrations (10 and 20 mg/kg/day) in a rabbit model showed a statistically insignificant effect on the PK profile of TAC. In consistence with our results, a study showed that TICA had no effect on PK parameters of cyclosporine under their study conditions (Teng *et al.,* 2014). In addition, two previous studies found that co-administration of pantoprazole and TAC in patients with liver transplant was associated with no significant effect on TAC serum levels (Lorf *et al.,* 2000; Bremer *et al.,* 2018). Furthermore, Homma and his collaborators in a further study showed that lansoprazole (a CYP3A4 inhibitor) significantly increased the blood concentrations of TAC when concomitantly administered in renal transplanted patients. In the same study and under the same conditions, they found no significant effect occurred on blood concentrations of TAC when lansoprazole was substituted with rabeprazole, which is a substrate of CYP3A4 (Homma *et al.,* 2002). Also, similar to our findings, Bifano and his colleagues found that daclatasvir (an antiviral drug used for treating hepatitis A virus and a substrate of both CYP3A4 and P-gp) had insignificant effects on the PK profile of TAC when a single dose was used for TAC and multiple doses of daclatasvir in healthy volunteers (Bifano *et al.,* 2015). In conclusion, we found that co-administration of TICA at different concentrations (10 and 20

mg/kg/day) in the rabbit model had no significant effect on the PK parameters of TAC.

## Conclusion

The present study demonstrated that concurrent use of TICA in the examined regimen had not influenced PK parameters of TAC. Further PK studies of TAC using TICA at higher doses and for a longer duration may be advised to be conducted before these results can be applied in patient care.

## Conflict of Interests:

None to declare

## Authors Contribution:

The authors were contributed to the paper writing, revision, and final approval.

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