Pharmacokinetic/Pharmacodynamic Analysis of Tacrolimus-Induced QT Prolongation in Guinea Pigs

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Recently, several reports of clinical cases of QT prolongation and *torsades de pointes*, associated with the use of tacrolimus (FK506), have come to light. We have previously demonstrated FK506-induced QT prolongation in guinea pigs [Minematsu T., *et al.*, *Life Sci.*, 65, PL197–PL202 (1999)]. We now examined the relationship between QTc prolongation and the pharmacokinetics of FK506 in guinea pigs, in order to evaluate the arrhythmogenicity of FK506 when compared with that of quinidine sulfate (QND). Thus, dose–response relationships for FK506 (0.01 or 0.1 mg/h/kg) or QND (30 mg/h/kg) were investigated during and after intravenous infusion and also following intravenous bolus administration of FK506 (0.2 mg/kg). The dose–response relationship between plasma drug concentration and QTc prolongation for FK506 and QND were subsequently analyzed using an effect compartment model. The pharmacodynamic parameters thus obtained were as follows: k_{E0} 2.72×10^{-4} (min^{-1}), E_{max} 27.1 (ms), EC_{50} 0.376 (ng/ml) for FK506; and k_{E0} 0.148 (min^{-1}), K 8.41 ($\text{ms} \cdot \text{ml}/\mu\text{g}$) for QND. The anti-clockwise hysteresis observed for FK506-induced QT prolongation was successfully analyzed by the present pharmacokinetic/pharmacodynamic model, which may provide a rational basis for developing a clinical dosing regimen to avoid possible QT prolongation induced by FK506.

Key words tacrolimus; quinidine; QT prolongation; electrocardiogram; arrhythmia; pharmacodynamic

Tacrolimus (FK506), a potent immunosuppressant widely used in organ transplantation,¹⁾ is currently undergoing clinical trials for use in the treatment of autoimmune diseases.²⁾ However, recent reports^{3–6)} suggest that FK506 is cardiotoxic. Especially, clinical cases of *torsades de pointes* and QT prolongation have been reported following treatment with FK506.^{7,8)} We recently reported that FK506 prolonged the QT interval in guinea pigs, and that there was an anti-clockwise hysteresis effect related to whole blood FK506 concentration.⁹⁾ However, quantitative analysis of FK506-induced QT prolongation has so far not been reported, thus the present study serves to address this issue.

Previously, in order to quantitatively estimate the risk of drug-induced QT prolongation, we have examined the pharmacokinetic–pharmacodynamic (PK/PD) relationship between drug concentration and QT prolongation in small experimental animals using various therapeutic agents.^{10–14)} Thus, in the present study we have performed a PK/PD analysis of FK506-induced QT prolongation in guinea pigs, using an effect compartment model.¹⁵⁾ As a positive control, we employed quinidine sulfate (QND), which has been attributed as the primary cause of drug-induced *torsades de pointes* in humans.¹⁶⁾

MATERIALS AND METHODS

Animals Male Hartley guinea pigs weighing 360–500 g were purchased from Nihon Ikagaku Dobutsu Shizai Kenkyusho (Tokyo, Japan) and kept with free access to food and water.

Materials FK506, as a solution, (Prograf® injection, 5 mg/ml) and QND, in powder form, were purchased from Fujisawa Pharmaceutical (Osaka, Japan) and Wako Pure Chemical Industries (Osaka, Japan), respectively. All other chemicals used were of reagent grade.

In Vitro Blood-to-Plasma Concentration Ratio of FK506 Initially the relationship between whole blood and

plasma concentrations of FK506, which is known to be non-linear¹⁷⁾ in humans,¹⁸⁾ rats,¹⁹⁾ and rabbits,^{20,21)} was established, in order to permit estimation of the plasma concentration of FK506 from the data for *in vivo* whole blood concentrations. Guinea pigs were anesthetized with a combination of urethane and α -chloralose (1.2 mg/kg and 30 mg/kg i.p., respectively). Subsequently, blood was collected from the carotid artery and EDTA 2Na was added at 1 mg/ml of whole blood. An aliquot of the collected blood was centrifuged at $4000 \times g$ for 4 min to determine the hematocrit value (*Ht*). Subsequently, after preincubation for 3 min at 37 °C, aliquots of the collected blood (990 μl) were spiked with 10 μl of FK506, yielding whole blood FK506 concentrations of 12.5, 25, 50, 100, 200, 400, and 800 ng/ml. Following this, the spiked blood samples were incubated for 30 min at 37 °C. Plasma and blood cells were then separated by centrifugation at 37 °C for 4 min at $1000 \times g$. Finally, the concentration of FK506 in both whole blood and plasma in each aliquot was determined, using a microparticle enzyme immunoassay (MEIA), as previously reported.⁹⁾ At steady state, the relationship between *Ht* and the concentrations of FK506 in whole blood (C_{WB}), and plasma (C_{P}) can be expressed as follows²⁰⁾:

$$C_{\text{WB}} = C_{\text{P}} + Ht \cdot \frac{B_{\text{max}} \cdot C_{\text{P}}}{K_{\text{d}} + C_{\text{P}}} \quad (1)$$

where B_{max} is the binding capacity, and K_{d} is the affinity constant. These parameters were obtained by fitting the observed data to Eq. (1) using a nonlinear least-squares regression program (MULTI).²²⁾

Pharmacokinetic Experiments Data from infusion experiments for whole blood FK506 concentrations and plasma QND concentrations were derived from our previous study.⁹⁾ For studies on the intravenous bolus administration of FK506, guinea pigs were anesthetized in the same manner described above, and restrained in a supine position. The trachea, right jugular vein, and right carotid artery were then

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cannulated with polyethylene tubing. A body temperature at $37.5 \pm 0.5^\circ\text{C}$ was maintained throughout the experiment. FK506 (0.2 mg/kg) was intravenously administered, through the jugular vein, as a diluted solution of 0.2 mg/ml FK506 in a physiological salt solution (NaCl 135 mM, NaHCO_3 11.9 mM, KCl 5.4 mM, CaCl_2 1.8 mM, MgCl_2 1.0 mM). Blood samples of 300 μl were collected from the carotid artery at 2, 5, 15, 30, 60, 120, 180, and 240 min for the bolus administration study, and a whole blood concentration of FK506 was subsequently determined by the MEIA method. In addition, plasma potassium concentrations were determined by the atomic absorption method, previously described by Hirota *et al.*¹²⁾

Pharmacodynamic Experiments Data from infusion experiments for FK506- and QND-induced QT prolongation were derived from our previous study.⁹⁾ In the intravenous bolus administration study, pharmacodynamic experiments were performed, without blood sampling, on groups of animals different from those used in the pharmacokinetic experiments, but using identical conditions. The electrocardiogram (ECG) was recorded and analyzed as previously described by Ohtani *et al.*¹⁰⁾ Briefly, signals from the bipolar lead II were amplified by means of an electric amplifier (AB-621G, Nihon-Koden, Tokyo, Japan), stored in a personal computer (PC-9801VX, NEC, Tokyo, Japan) via an A/D converter (Analog-Pro Jr., Canopus Electric, Kobe, Japan) and analyzed using WAVE MASTER II software (Canopus Electric, Kobe, Japan). ECG parameters were derived from the average wave shape of 10 s recordings. For the determination of QT prolongation, we exploited the change in QTc intervals (i.e. QT intervals corrected by Bazett's formula²³⁾) since, in the case of guinea pig ECGs, QT intervals have been reported to be affected by heart rates.²⁴⁾

Pharmacokinetic Analysis In the case of FK506, a 3-compartment model, which assumes rapidly equilibrating but saturable intra-blood-cellular binding (Fig. 1), was employed to enable analysis of the time profile of whole blood FK506 concentration. In the 3-compartment model, the following equations were indicated:

Infusion study ($I=0$ after 90 min),

$$\frac{dC_1}{dt} = \frac{I}{V_1} + k_{12} \cdot (C_2 - C_1) + k_{13} \cdot (C_{3u} - C_1) - k_{10} \cdot C_1, \quad C_1(0)=0 \quad (2-1)$$

$$\frac{dC_2}{dt} = k_{12} \cdot (C_1 - C_2), \quad C_2(0)=0 \quad (2-2)$$

$$\frac{dC_3}{dt} = k_{31} \cdot (C_1 - C_{3u}), \quad C_3(0)=0 \quad (2-3)$$

$$C_{3u} = \frac{-(K_d + B_{\max} - C_3) + \sqrt{(K_d + B_{\max} - C_3)^2 + 4 \cdot K_d \cdot C_3}}{2} \quad (2-4)$$

$$C_{WB} = (1 - Ht) \cdot C_1 + Ht \cdot C_3 \quad (2-5)$$

where t is time after drug administration; C_1 , C_2 , and C_3 are FK506 concentrations in the central compartment, peripheral compartment, and blood cell compartment, respectively; $C_1(0)$, $C_2(0)$, and $C_3(0)$ are C_1 , C_2 , and C_3 before the start of drug administration, respectively; C_{3u} is the unbound FK506 concentration in blood cell compartment; k_{10} , k_{12} , k_{21} , k_{13} , and k_{31} are rate constants; V_1 is the distribution volume of the central compartment; and I is an infusion rate. The above equations were obtained by reducing the distribution volume

Fig. 1. PK/PD Model Applied to FK506-Induced QT Prolongation

See text for abbreviations. A bidirectional arrow denotes instantaneous equilibrium, and a single directional solid arrow denotes a non-instantaneous equilibrium process.

of the peripheral compartment and blood cell compartment with equations, i.e. $k_{12} \cdot V_1 = k_{21} \cdot V_2$, $k_{13} \cdot V_1 = k_{31} \cdot V_3$:

Bolus study,

$$\frac{dC_1}{dt} = k_{12} \cdot (C_2 - C_1) + k_{13} \cdot (C_{3u} - C_1) - k_{10} \cdot C_1, \quad C_1(0) = \frac{\text{Dose}}{V_1} \quad (3)$$

Other equations for dC_2/dt , dC_3/dt , C_{3u} , and C_{WB} are the same as Eqs. (2-2), (2-3), (2-4), and (2-5), respectively. The parameters of k_{10} , k_{12} , k_{21} , k_{13} , k_{31} , and V_1 were obtained by simultaneous fitting of the infusion and bolus data to the above pharmacokinetic model using the MLAB computer program (Civilized Software, Inc., Bethesda, U.S.A.). In the case of QND, a 2-compartment open model was employed to enable analysis of the time profile of plasma QND concentration. Pharmacokinetic parameters were obtained by fitting of the observed data to the pharmacokinetic model using MLAB.

PK/PD Analysis of QTc Prolongation The effect of a given drug at each time-point was expressed in terms of the change from the value prior to drug administration. The plasma concentration at each time-point, employed here as the input function, was the value calculated from the pharmacokinetic parameters obtained above. Pharmacodynamic analyses of FK506 and QND were conducted using the effect compartment model (Fig. 1),¹⁵⁾ as delays in QTc prolongation were observed for both drugs. The drug concentration in the effect compartment (C_E) is described by Eq. (4):

$$\frac{dC_E}{dt} = k_{E0} \cdot (C_1 - C_E) \quad (4)$$

where k_{E0} denotes the elimination rate constant from the effect compartment.

In the case of FK506, the $E_{\max}$ model described by the following differential equation was employed to relate C_E to the effect (E), since a saturation of the effect with respect to C_E was observed:

$$\frac{dE}{dt} = \frac{EC_{50} \cdot E_{\max}}{(EC_{50} + C_E)^2} \cdot \frac{dC_E}{dt} \quad (5)$$

where $E_{\max}$ and EC_{50} are the maximal effect and the concentration which gives the half-maximal effect, respectively. The pharmacodynamic parameters of k_{E0} , $E_{\max}$, and EC_{50} were

obtained by simultaneous fitting of the infusion and bolus data to Eqs. (4) and (5), using MLAB.

The effect compartment model was also applied to QND. However, in this case a linear relationship between E and C_E as described by Eq. (6) was applied, since the $E_{\max}$ model failed to satisfactorily describe this relationship:

$$\frac{dE}{dt} = K \cdot \frac{dC_E}{dt} \quad (6)$$

where K is a slope constant. The pharmacodynamic parameters of k_{E0} and K were obtained by fitting the observed data to Eqs. (4) and (6), using MLAB.

RESULTS

The Blood-to-Plasma Concentration Ratio for FK506

Fig. 2. *In Vitro* Assay of the Relationship between Plasma FK506 Concentration and Whole Blood FK506 Concentration in Guinea Pigs (●), Rats (△), and Humans (□)

Data for rats and humans were derived from previous studies.^{18,19} The line represents a best-fit of the data to Eq. (1).

The relationship between whole blood and plasma concentrations of FK506 showed a nonlinear profile (Fig. 2), probably as a result of a saturable distribution property of FK506 into blood cells. Ht was found to be 0.452 and the values of $B_{\max}$ and K_d were estimated to be 556 ± 20 ng/ml and 7.85 ± 1.73 ng/ml, respectively (estimate $\pm$ S.D.).

Pharmacokinetics of FK506 and QND in Guinea Pigs

Figures 3A and 3B illustrate whole blood concentrations of FK506 with respect to time after infusion and bolus administration, respectively. Figures 3C and 3D represent the time courses of simulated plasma concentrations of FK506 (C_1), with respect to time after infusion and bolus administration, respectively. The pharmacokinetic parameters were presented in Table 1. In the FK506 bolus study, no significant change in plasma potassium concentration was observed, i.e. 3.46 ± 0.40 mEq (mean $\pm$ S.E.M.), before drug administration compared to 3.49 ± 0.44 mEq at 240 min after administration ($n=6$). The time course of plasma QND concentration was accurately predicted by the 2-compartment open model (Fig. 4 and Table 1).

Pharmacodynamics of QT Prolongation by FK506 An administration rate-dependent increase in the QTc interval was observed during the constant intravenous infusion of FK506 (Fig. 5A). Prolongation of the QTc interval persisted until 150 min after termination of the infusion. Similarly, following intravenous bolus administration of FK506, prolongation of the QTc interval also persisted, for as long as 240 min after administration (Fig. 5B). The relationship between the plasma concentration of FK506 and the change in QTc interval after infusion showed an anti-clockwise hysteresis (Fig. 6).

Pharmacodynamics of QT Prolongation by QND In

Fig. 3. Time Courses of Blood FK506 Concentration in Guinea Pigs

A. Whole blood concentrations of FK506 during and after intravenous FK506 infusion at 0.01 mg/h/kg (△), 0.1 mg/h/kg (○). B. Whole blood concentrations of FK506 after intravenous bolus administration of FK506 (0.2 mg/kg). C. Calculated plasma concentrations of FK506 during and after intravenous FK506 infusion at 0.01 mg/h/kg, and 0.1 mg/h/kg. D. Calculated plasma concentrations of FK506 after intravenous bolus administration of FK506 (0.2 mg/kg). Data are expressed as the mean $\pm$ S.E.M. of 5 animals. A best-fit line to a 3-compartment model is shown for each graph. Data for whole blood FK506 concentrations in the infusion study were derived from Ref. 9.

Table 1. Pharmacokinetic Parameters of FK506 and QND in Guinea Pigs

Parameters	FK506	QND
k_{10} (min^{-1})	0.0285 [0.0245, 0.0325]	0.152 [0.099, 0.205]
k_{12} (min^{-1})	0.0523 [0.0513, 0.0533]	0.431 [0.384, 0.478]
k_{21} (min^{-1})	0.019 [0.0137, 0.0243]	0.0142 [0.0107, 0.0177]
k_{13} (min^{-1})	0.0931 [0.0662, 0.110]	—
k_{31} (min^{-1})	7.46 [5.85, 9.07]	—
V_1 (ml/kg)	1140 [1060, 1220]	239 [218, 260]

See text for abbreviations. Parameters were obtained by fitting the data for drug concentrations to each pharmacokinetic model. Values are expressed as the estimate [68% confidence interval].

Fig. 4. Time Course of Plasma Concentration of QND in Guinea Pigs during and after Intravenous Infusion of QND at 30 mg/h/kg (○)

Data are expressed as the mean $\pm$ S.E.M. of 5 animals. A best-fit line to a 2-compartment open model is shown. Data for plasma QND concentrations were derived from Ref. 9.

Fig. 5. Time Courses of the Change in QTc Interval in Guinea Pigs after Administration of FK506

A. The change in QTc interval during and after intravenous infusion of vehicle (□), FK506 at 0.01 mg/h/kg (Δ), and FK506 at 0.1 mg/h/kg (○). B. The change in QTc interval after intravenous bolus administration of vehicle (□), and FK506 (0.2 mg/kg) (○). Data are expressed as the mean $\pm$ S.E.M. of 4–5 animals. A best-fit line to the effect compartment model is shown for each graph. Some of the data were derived from Ref. 9.

Fig. 6. Relationship between the Plasma FK506 Concentration and the Change in QTc Interval in Guinea Pigs

A. Intravenous infusion of FK506 at 0.01 mg/h/kg (Δ), and 0.1 mg/h/kg (○). B. Intravenous bolus administration of FK506 (0.2 mg/kg). Data are expressed as the mean $\pm$ S.E.M. of 5 animals. The therapeutic ranges for plasma FK506 concentration, corresponding to a whole blood FK506 concentration range of 5–20 ng/ml, were calculated using Eq. 1. Each arrow indicates an elapse of time. A best-fit line to the effect compartment model is shown for each graph. Some of the data were derived from Ref. 9.

Fig. 7. Time Courses of the Change in QTc Interval in Guinea Pigs during and after Intravenous Infusion of Vehicle (□) and QND at 30 mg/h/kg (○)

Data are expressed as the mean $\pm$ S.E.M. of 5 animals. A best-fit line to the effect compartment model is shown. Some of the data were derived from Ref. 9.

Fig. 8. Relationship between the Change in QTc Interval and the Plasma QND Concentration in Guinea Pigs

Data are expressed as the mean $\pm$ S.E.M. of 5 animals. Each arrow indicates an elapse of time. A best-fit line to the effect compartment model is shown. Some of the data were derived from Ref. 9.

Table 2. Pharmacodynamic Parameters of FK506 and QND

Parameters	FK506	QND
k_{E0} (min^{-1})	2.72×10^{-4} [1.66×10^{-4} , 7.48×10^{-4}]	0.148 [0.126, 0.170]
E_{max} (ms)	27.1 [25.8, 28.4]	—
EC_{50} (ng/ml)	0.376 [0.289, 0.489]	—
K ($\text{ms} \cdot \text{ml}/\mu\text{g}$)	—	8.41 [8.04, 8.79]

See text for abbreviations. The parameters were derived using an effect compartment model. Values are expressed as the estimate [68% confidence interval].

contrast to FK506, QND administration resulted in a QTc interval that returned to basal levels 150 min after termination of the infusion (Fig. 7). The relationship between plasma concentration of QND and the change in QTc interval after infusion also showed an anti-clockwise hysteresis (Fig. 8).

PK/PD Analysis of QT Prolongation The pharmacodynamic parameters are presented in Table 2. It can be seen that the k_{E0} value for FK506 is far lower than that for QND. The best-fit lines in Figs. 5—8 were generated using the effect compartment model.

DISCUSSION

In this study, the effect of FK506 on the QT interval was investigated in guinea pigs in a quantitative manner, since QT prolongation associated with FK506 has been reported clinically^{7,8)} and experimentally.⁹⁾

A delay of QT prolongation with respect to plasma concentration was observed for both FK506 and QND. Therefore, the effect compartment model¹⁵⁾ was applied to analyze the relationship between the plasma concentrations of these drugs and QT prolongation. Although the k_{E0} value for FK506 appears to exceed the range of values for the parameters that could feasibly be determined under the present time scale, the QT prolonging effect of FK506 clearly persisted for at least 4 h. In any event, it is of note that the QT prolongation induced by FK506 persisted for much longer than that induced by QND. Selection of an appropriate procedure for the modeling of PK/PD data should, if possible, be based on the mechanism by which a drug induces its response. However, the effect compartment model is applicable not only to a drug which is slow to transfer to its effect site but also to a drug with an active metabolite (e.g. QND^{15,25)}), or with an indirect response. In this regard, the effect compartment model is a general and practical model for analyzing the PK/PD relationship to anti-clockwise hysteresis. Whereas the delay of QND-induced QT prolongation is known to be due to the effects of its metabolites,^{15,25–29)} that of FK506 remains open to question.⁹⁾ Therefore, we employed the effect compartment model to analyze the PK/PD of FK506- and QND-induced QT prolongation rather than using mechanism-based models such as an active metabolite model³⁰⁾ or an indirect response model.^{31–33)}

QND induced significant QTc prolongation in guinea pigs at a plasma concentration range of 3—7 $\mu\text{g}/\text{ml}$, which corresponds with the therapeutic range. This result agrees well with the high arrhythmogenic activity of QND observed clinically. In comparison, the therapeutic range of FK506 is 5—20 ng/ml,^{34,35)} when measured as whole blood trough concentration. Since the blood-to-plasma concentration ratios of FK506 in human and guinea pigs were almost equal, as evidenced by this study and that of Nagase *et al.*¹⁸⁾ (Fig. 2), we

estimated that the therapeutic plasma concentration of FK506 was 0.154—0.654 ng/ml at an Ht of 0.452, using Eq. 1. However, in some clinical cases of transplantation, the Ht is usually reduced to 0.2 to decrease the viscosity of blood.³⁶⁾ In such a case, an FK506 concentration of 5—20 ng/ml whole blood would give a plasma concentration of 0.343—1.56 ng/ml, at which concentration significant QTc prolongation was observed in the current study. Therefore, we propose that FK506 might induce QT prolongation within its therapeutic range in clinical use. It is of note that blood distribution property and Ht should be important physiological factors in predicting the PK/PD of FK506, as well as in predicting that of cyclosporine A.^{37–40)}

It is desirable to directly determine the plasma concentration of FK506, since the plasma concentration is likely to relate more directly to the effects of FK506 than the whole blood concentration. In the present *in vivo* study, the plasma concentration of FK506 was simulated from the whole blood concentration by means of the *in vitro* blood-to-plasma concentration ratio and 3-compartment model above. In humans,¹⁸⁾ rats¹⁹⁾ and rabbits,^{20,21)} the distribution of FK506 into blood cells is extensive and saturable and the present study showed that the drug also exhibited a similar property in guinea pigs (Fig. 2). However, the distribution of FK506 into blood cells is reported to be temperature-dependent.⁴¹⁾ In clinical settings, whole blood concentrations of FK506 are used for therapeutic drug monitoring,³⁴⁾ and a small sample volume is sufficient for monitoring whole blood concentration. For these reasons, monitoring whole blood concentrations appears far more advantageous and practical than monitoring plasma concentrations.

In conclusion, FK506-induced QTc prolongation, which we previously reported for the first time in guinea pigs,⁹⁾ was successfully analyzed using the effect compartment model. Plasma concentrations of the drug, which are more relevant than whole blood concentrations for investigating such pharmacological effects as adverse reactions, were employed for pharmacokinetically meaningful comparison between guinea pigs and humans. The present model may provide a rational basis for developing clinical strategies to avoid possible QT prolongation resulting from either an overdose of FK506, metabolic inhibition by a concomitantly administered drug, or decreases in Ht . Further study is required in order to fully elucidate the mechanism by which FK506 induces QT prolongation.

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