Research Square

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Association between itraconazole formulations and blood tacrolimus concentrations in lung transplant recipients: a retrospective observational study

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Abstract

Background

Tacrolimus (TCR) is an essential immunosuppressant to prevent organ rejection in lung transplant (LT) recipients. Some concomitant drugs challenge TCR management through drug–drug interactions. Itraconazole (ITCZ) is a potent cytochrome P450 inhibitor that increases the blood TCR levels in a concentration-dependent manner. ITCZ has some dosage forms: one is a capsule (ITCZ-CP) with low bioavailability and another is an oral solution (ITCZ-OS) with improved intestinal absorption via a solubilizing agent. Therefore, switching from ITCZ-OS to ITCZ-CP should decrease serum ITCZ concentrations and alter TCR levels. However, there is no evidence on the extent to which changes in ITCZ formulation affect blood TCR concentrations. This study aimed to quantitatively clarify the effect of switching the ITCZ formulation on the TCR concentration in LT recipients.

Methods

Single-center retrospective analyses were conducted at The University of Tokyo Hospital between January 2018 and December 2024. Blood TCR and serum ITCZ concentrations were analyzed from 7 days before through 21 days after the ITCZ formulation was switched.

Results

Switching ITCZ formulations lowered the median TCR concentration-to-dose (C/D) ratios from 318.3 (ng/mL)/(mg/kg/day) to 237.3 (ng/mL)/(mg/kg/day) ($P < 0.001$). Additionally, the median serum total ITCZ concentrations across the three subjects decreased from 773.3 ng/mL to 324.9 ng/mL ($P < 0.05$).

Conclusions

Switching ITCZ formulations from ITCZ-OS to ITCZ-CP decreased the TCR C/D ratios in LT recipients. Frequent monitoring of blood TCR concentrations is required to avoid the critical consequences of low blood TCR concentrations.

Background

Tacrolimus (TCR) is a key immunosuppressant for lung transplant (LT) recipients to prevent rejection. Despite its indispensability in LT, excessive blood TCR levels can harm recipients by causing severe adverse events such as opportunistic infection and kidney injury. Therefore, it is important to maintain blood TCR concentrations within therapeutic ranges by adjusting dosage based on therapeutic drug monitoring (TDM) results.

Among the several factors affecting TCR pharmacokinetics, the activities of cytochrome P450s (CYPs), which play pivotal roles in TCR metabolism, are clinically important in terms of drug–drug interactions. Azole antifungal drugs are well-known CYP inhibitors. They can increase the blood concentration of concomitant CYP substrate drugs, including TCR, increasing the risk of adverse events. However, azoles are necessary to prevent fatal pulmonary aspergillosis in LT recipients [1]. Itraconazole (ITCZ) is a clinically used azole that is effective against Aspergillus. However, ITCZ and its metabolites (hydroxy-ITCZ, keto-ITCZ, and N-desalkyl-ITCZ) strongly inhibit CYPs, especially CYP3A isoforms [2]. Previous studies have revealed that ITCZ inhibits TCR metabolism in vitro [3] and increases the concentration-to-dose (C/D) ratio of TCR [4] in a concentration-dependent manner. Given the concentration-dependent action of ITCZ on TCR pharmacokinetics, blood TCR levels should be carefully monitored not only at the start of ITCZ treatment but also when it is discontinued. In the latter case, blood TCR concentrations could be lowered, increasing the risk of rejection. Thus, to maintain blood TCR levels within appropriate ranges, changes in ITCZ pharmacokinetics should be considered.

ITCZ exhibits poor water solubility, which restricts its intestinal absorption. Hence, the bioavailability of the initially developed ITCZ formulation, ITCZ capsule (ITCZ-CP), is low (55%) [5]. Because the water solubility of ITCZ increases under acidic conditions, ITCZ-CP should be administered immediately after meals, when gastric acid secretion is active. However, in LT recipients, gastric acid secretion inhibitors such as H2 blockers and proton pump inhibitors are frequently coadministered to prevent pulmonary complications arising from reflux esophagitis, thus contributing to lower ITCZ bioavailability [6, 7]. To resolve these issues, an oral ITCZ solution (ITCZ-OS) has been developed. The bioavailability of ITCZ-OS is up to 30% higher than that of ITCZ-CP when hydroxypropyl-beta-cyclodextrin (HP-β-CD) is added as a solubilizing agent [8]. On the other hand, HP-β-CD could cause gastrointestinal adverse events such as diarrhea and nausea [9], which might lead to difficulties in continuing ITCZ-OS treatment. In such cases, switching from ITCZ-OS to ITCZ-CP is sometimes necessary, which decreases the bioavailability of ITCZ. Given the concentration-dependent action of ITCZ on TCR pharmacokinetics, this formulation switch should decrease TCR blood levels. However, there is no evidence to show the extent to which these formulation changes would affect blood TCR concentrations.

Therefore, this study conducted retrospective medical record analyses to clarify the effect of switching ITCZ formulations on blood TCR concentrations in LT recipients.

Methods

Patients

We conducted a single-center retrospective analysis, in which LT recipients who met the following criteria were included: 1) LTs performed at The University of Tokyo Hospital between January 2018 and December 2024. 2) LT recipients who received simultaneous administration of TCR and ITCZ. 3) ITCZ formulations were switched from ITCZ-OS to ITCZ-CP during hospitalization. LT recipients discharged from the hospital within 21 days of switching ITCZ formulations were excluded.

A total of 24 LT recipients were analyzed. Serum ITCZ concentrations were measured in three recipients for research purposes after written informed consent was obtained. The target TCR trough concentration after LT was 10–13 ng/mL for the initial three months, and 8–11 ng/mL thereafter.

Data collection

Data on the blood TCR concentration, age, body weight, blood sampling date, and concomitant drug use were obtained from the electronic medical records of The University of Tokyo Hospital. Blood TCR concentrations were analyzed from 7 days before through 21 days after the ITCZ formulation was switched.

Blood TCR concentrations were measured using a chemiluminescent immunoassay (CLIA, ARCHITECT Tacrolimus Assay, Abbott; Abbott Park, Illinois, USA) (until June 13, 2021) and an affinity column immunoassay (ACMIA, Dimension Tacrolimus Flex Reagent Cartridge, Siemens Healthineers; Erlangen, Munich, Germany) (from June 14, 2021).

Serum ITCZ concentrations and their metabolites were measured in residual samples using liquid chromatography tandem mass spectrometry (LC-MS/MS) on an ACQUITY UPLC-Quattro Premier XE system (Waters; Milford, Massachusetts, USA). The detailed methods for the LC-MS/MS analyses are described in the Supplementary Materials.

The TCR C/D ratios and total serum ITCZ concentrations during the ITCZ-OS and ITCZ-CP periods were calculated as average values from day − 7 to day 0 (switching date) and from day 14 to day 21, respectively.

The administration status of concomitant drugs that might affect TCR and ITCZ pharmacokinetics were monitored as follows: drugs that would induce or inhibit CYP3A and/or P-glycoprotein, such as rifampicin, antiepileptic drugs (phenytoin, carbamazepine, and phenobarbital), azole antifungals (voriconazole, fluconazole, posaconazole, and isavuconazole), macrolide antibiotics (clarithromycin and erythromycin), calcium channel blockers (diltiazem and verapamil), and ritonavir: antacid agents, such as proton pump inhibitors (omeprazole, lansoprazole, esomeprazole, rabeprazole, and vonoprazan), and H2 blockers (cimetidine, famotidine, nizatidine, roxatidine, and lafutidine).

Materials

Bifonazole was purchased from LKT Laboratories (St. Paul, Minnesota, USA). Itraconazole (ITCZ) and keto-ITCZ were purchased from Santa Cruz Biotechnology (Dallas, Texas, USA), and hydroxy-ITCZ and N-desalkyl-ITCZ were purchased from Toronto Research Chemicals (North York, Ontario, Canada).

Liquid chromatography tandem mass spectrometry analyses

For ITCZ measurements, residual serum samples from clinical biochemical examinations were collected and stored at − 80°C until liquid chromatography-tandem mass spectrometry (LC-MS/MS) analyses. Twenty microliters of serum were deproteinized with four volumes of methanol containing 10 ng/mL bifonazole (internal standard) and centrifuged at 10,000 rpm for 10 min at room temperature. One hundred microliters of the supernatant were used for LC-MS/MS analyses.

LC-MS/MS analyses were conducted using an ACQUITY UPLC-Quattro Premier XE system coupled to an ACQUITY Ultra-Performance LC System with an integral auto-injector (Waters, Milford, Massachusetts, USA). Five microliters of each sample were injected into an AQUITY UPLC BEH C18 column (1.7 μm, 2.1 × 150 mm; Waters) and subjected to separation. The Premier XE spectrometer was operated in electrospray ionization (ESI)-MS/MS multiple reaction monitoring mode at a source temperature of 150°C and a desolvation temperature of 450°C. Formic acid (0.1% (v/v)) in LC-grade ultrapure water (solvent A) and in LC-grade acetonitrile (solvent B), respectively, were used as mobile phases. The samples were separated by gradient LC conditions at a flow rate of 0.3 mL/min as follows: 0–2 min: 5% B, 2–4 min: 5–95% B, 4–7 min: 95% B, 7–7.5 min: 95–5% B, 7.5–9 min: 5% B. Column temperature was maintained at 50°C. The MS conditions (parent mass > daughter mass, cone voltage, and collision energy) were as follows: bifonazole—311.37 > 243.20, 10 V, 30 eV; ITCZ—705.30 > 392.48, 45 V, 36 eV; keto-ITCZ—719.36 > 406.50, 50 V, 38 eV; hydroxy-ITCZ—721.36 > 408.50, 55 V, 36 eV; N-desalkyl-ITCZ—649.33 > 336.40, 45 V, 38 eV. All compounds were analyzed in positive ion mode. Data analyses were performed using MassLynxNT software (version 4.1; Waters). The obtained area values of ITCZ, keto-ITCZ, hydroxy-ITCZ, and N-desalkyl-ITCZ were normalized to those of bifonazole and used for calculations. As the levels of keto-ITCZ were below the limit of quantification, the total ITCZ concentrations were determined as the sum of ITCZ, hydroxy-ITCZ, and N-desalkyl-ITCZ concentrations.

Statistical Analyses

Differences in the TCR C/D ratios and serum total ITCZ concentrations before and after switching formulations were assessed using the Wilcoxon signed-rank test. The correlation between the TCR C/D ratios and serum total ITCZ concentrations was assessed using the Pearson's correlation analysis. P values less than 0.05 were considered statistically significant. Statistical analyses were performed using the JMP software version 18 (SAS Institute Inc., Tokyo, Japan).

Results

Study Population

Medical records of 24 LT recipients (9 males and 15 females) were analyzed. All subjects were administered 200 mg/day of ITCZ regardless of its formulation. The median age of the subjects was 50 years (range: 18–65 years), and the median body weight was 46.7 kg (range: 30.0–78.9 kg). All subjects were administered antacid agents during the observation periods: 19 were proton pump inhibitors, and 5

were H_2 blockers. Although one subject took a macrolide, its effect was expected to be limited because the macrolide remained ingested throughout the observation period. The reasons for switching ITCZ formulations were diarrhea (n = 10), nausea (n = 9), and adherence-related reasons (n = 5).

Changes in blood TCR concentrations and C/D ratios after ITCZ formulation switching

The target TCR trough concentrations were 10–13 ng/mL for the initial three post-transplant months and 8–11 ng/mL thereafter. Fifteen subjects were in the former period, and 9 were in the latter period. TCR trough concentrations temporarily decreased after switching ITCZ formulations, whereas dose adjustment successfully maintained the TCR trough levels within the target ranges during the observation period (Fig. 1A, B). Switching ITCZ formulations lowered the median TCR C/D ratios from 318.3 (ng/mL)/(mg/kg/day) [range: 58.4–2814 (ng/mL)/(mg/kg/day)] to 237.3 (ng/mL)/(mg/kg/day) [range: 37.5–1972 (ng/mL)/(mg/kg/day)] ($P < 0.001$; Fig. 1C), that is, TCR C/D ratios decreased to 62.0% after switching from ITCZ-OS to ITCZ-CP (range: 25.6–131.7%, $P < 0.001$; Fig. 1D). No acute rejections were observed during the observation period.

Correlations between ITCZ concentrations and TCR C/D ratios

To examine whether the decrease in TCR C/D ratios after switching ITCZ formulations was related to changes in ITCZ levels, serum total ITCZ concentrations, defined as the sum of ITCZ and its major metabolites, were measured in the three subjects. Serum total ITCZ concentrations decreased soon after the ITCZ formulation was switched (Fig. 2A), with values decreasing significantly from 773.3 ng/mL (median; range: 603.5–901.8 ng/mL) to 324.9 ng/mL (median; range: 26.5–427.4 ng/mL) ($P < 0.05$). Positive correlations were observed between serum total ITCZ concentrations and TCR C/D ratios in all three subjects (Fig. 2C–E).

Discussion

This study showed that both serum total ITCZ concentrations and TCR C/D ratios significantly decreased after the ITCZ formulations were switched from ITCZ-OS to ITCZ-CP. Clear positive correlations were observed between serum total ITCZ concentrations and TCR C/D ratios. Given that ITCZ and its metabolites are potent inhibitors of CYP3A, which plays a major role in TCR metabolism, the correlations suggest that switching ITCZ formulations from ITCZ-OS to ITCZ-CP would decrease TCR C/D ratios by reducing serum total ITCZ concentrations. This is supported by a previous report on the correlation between ITCZ concentrations and TCR C/D ratios [4].

This study observed that TCR trough concentrations decreased to below the lower limit of the target ranges shortly after switching ITCZ formulations. However, frequent TDM of TCR enabled us to prevent

critical falls in blood TCR concentration, which could explain why no recipient experienced acute rejection during the observation period. Decrease in TCR concentration in the early post-transplantation period increases the risk of rejection [10]. Therefore, maintaining the target TCR concentration is extremely important, and frequent monitoring of TCR concentrations is required when serum ITCZ levels are affected.

Preventing pulmonary aspergillosis after LT is crucial for the long-term survival of LT recipients [11]. A previous report suggested that a serum ITCZ concentration of at least 500 ng/mL was required to prevent pulmonary aspergillosis [12]. In our study, ITCZ concentrations exceeded more than 500 ng/mL in two of the three subjects during the ITCZ-OS period. Nevertheless, after switching ITCZ formulations from ITCZ-OS to ITCZ-CP, the serum total ITCZ concentrations decreased to levels insufficient to prevent pulmonary aspergillosis in all cases. Because the intestinal absorption of ITCZ-CP requires dissolution by gastric acid, the drastic decrease in serum total ITCZ concentrations during the ITCZ-CP period may be caused by concomitant antacids, which are necessary for LT recipients to prevent pulmonary complications from reflux esophagitis [13]. Fortunately, none of the recipients experienced pulmonary aspergillosis during the observation period, however, other anti-Aspergillus azoles, such as voriconazole and posaconazole, whose intestinal absorption does not require gastric acid, could be alternatives when continuing ITCZ-OS is difficult in antacid administered LT recipients [14].

This study has some limitations associated with the study design. Because this was a retrospective study that mainly analyzed the electronic medical records of hospitalized patients, it was difficult to evaluate the long-term effects of temporal falls on blood TCR concentrations. Additionally, we obtained limited data on serum ITCZ concentrations because a few residual samples were available. Further long-term prospective studies are required to clarify the clinical impact of decreased blood TCR concentrations after switching to ITCZ formulations.

Conclusions

In conclusion, switching ITCZ formulations from ITCZ-OS to ITCZ-CP decreased the TCR C/D ratios in antacid-administered LT recipients. In these cases, frequent monitoring of blood TCR concentrations is recommended to avoid the critical consequences of low blood TCR levels.

Abbreviations

CYP
cytochrome P450
C/D
concentration to dose
HP-β-CD
hydroxypropyl-beta-cyclodextrin
ITCZ

itraconazole
CP
capsule
OS
oral solution
LC-MS/MS
liquid chromatography tandem mass spectrometry
LT
lung transplant
TCR
tacrolimus
TDM
therapeutic drug monitoring

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and its amendments, with approval from the Ethics Committee of The University of Tokyo Hospital (Approval Numbers 2023122NI and 2529). Written informed consent was obtained from all patients included.

Clinical trial number

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Author Contribution

H Masaki: conceptualization, data curation, formal analysis, funding acquisition, investigation, visualization, writing – original draft, writing – review and editing. H Miyata: data curation, formal analysis, investigation, supervision, validation, visualization, writing – original draft, writing – review and editing. MK: resources, supervision, writing – review and editing. CK: resources, supervision, writing – review and editing. MS: resources, supervision, writing – review and editing. TT: project administration, supervision, writing – review and editing. All authors read and approved the final manuscript.

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Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Figures

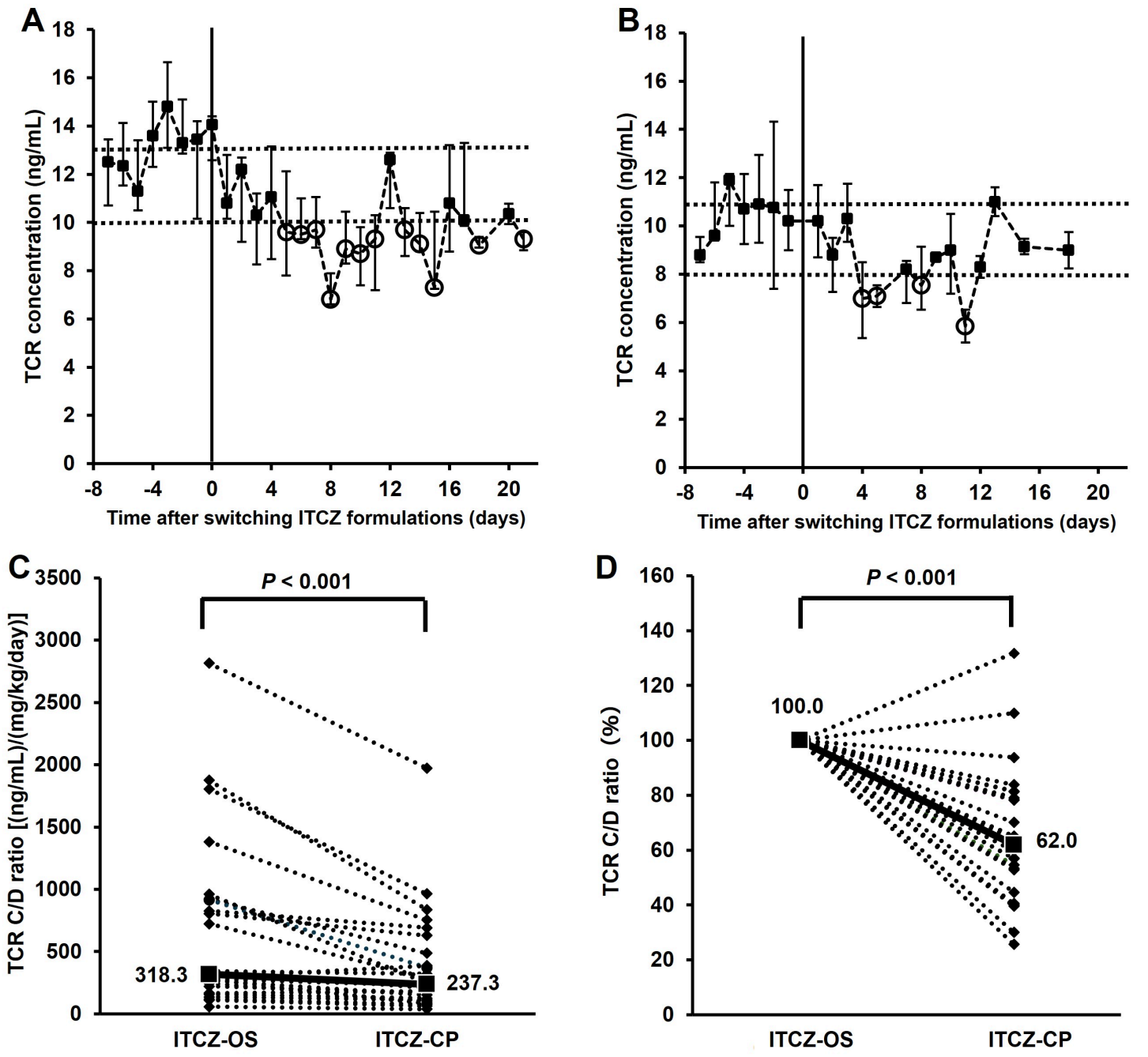

Figure 1

Changes in blood TCR trough concentrations and TCR C/D ratios after switching ITCZ-OS to ITCZ-CP

(A, B) Time-dependent changes in TCR trough concentrations. ITCZ formulations were switched from ITCZ-OS to ITCZ-CP on day 0. Each plot shows the median TCR concentration at the time point. The error bars mean the interquartile ranges. The target TCR trough concentration after LT was 10–13 ng/mL (A) for the initial three months, and 8–11 ng/mL (B) thereafter. The horizontal dotted lines indicate the upper and lower limits of the target concentrations. n = 3–6 (A), and 3–10 (B). (C, D) Changes in TCR C/D ratios. The solid lines show the median changes. n = 24

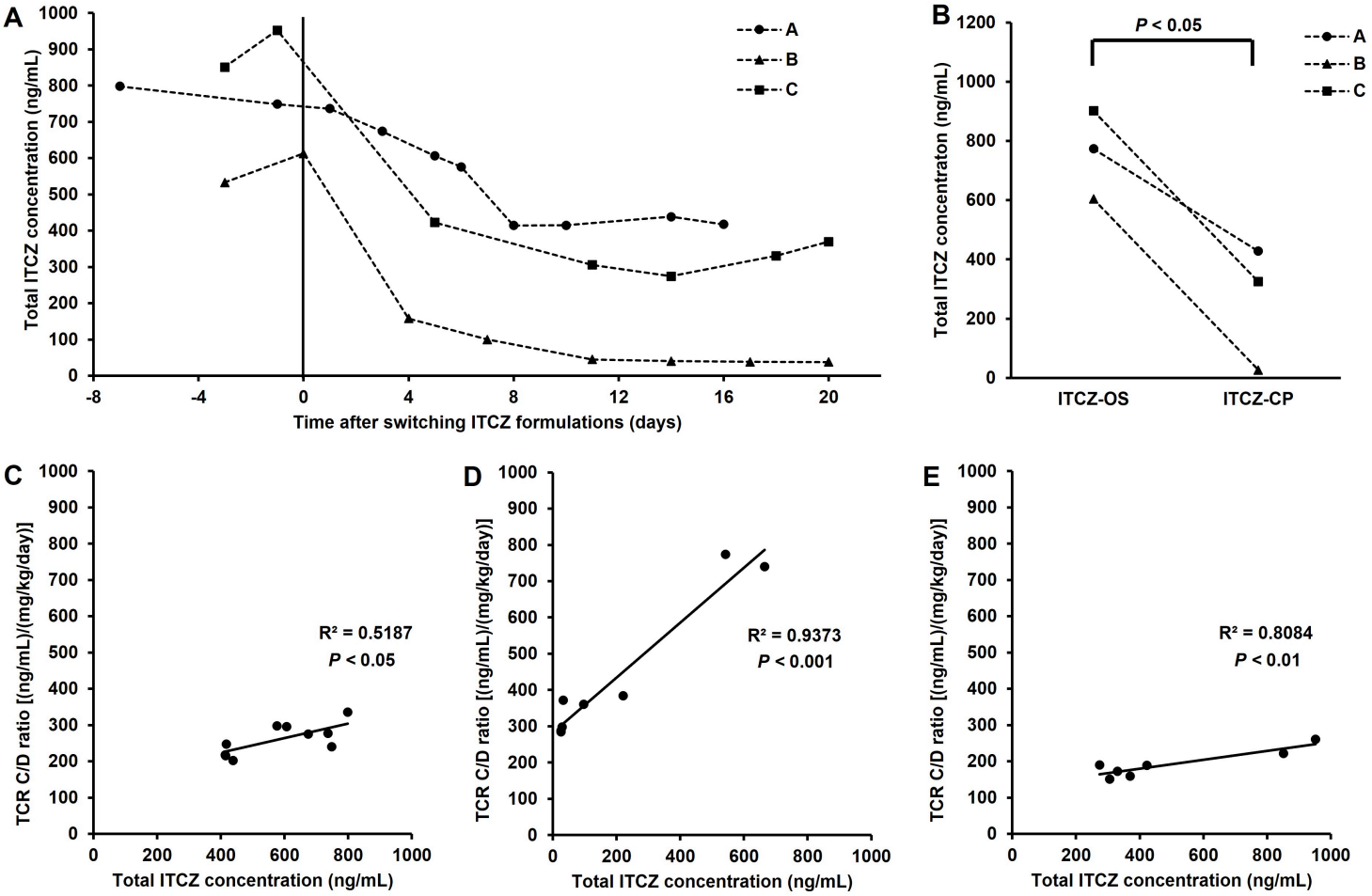

Figure 2

Relationship between serum total ITCZ concentrations and TCR C/D ratios

(A) The temporal changes in serum total ITCZ concentrations. ITCZ formulations were switched from ITCZ-OS to ITCZ-CP on day 0. (B) The average serum total ITCZ concentrations in ITCZ-OS and ITCZ-CP periods. The concentrations were calculated as the averages from days -7 to 0 for ITCZ-OS periods, and from days 14 to 21 for ITCZ-CP period. (C–E) The correlations between serum total ITCZ concentrations and TCR C/D ratios. (C) Subject A. (D) Subject B. (E) Subject C. Since the levels of keto-ITCZ were below the limit of quantification, total ITCZ concentrations were determined as the sum of ITCZ, hydroxy-ITCZ, and N-desalkyl-ITCZ concentrations.