Pediatric Transplantation

WILEY

ORIGINAL ARTICLE OPEN ACCESS

Precision Monitoring of Immunosuppressive Agent Concentrations in Cardiac Tissue of Pediatric Heart Transplant Recipients

Kazuhisa Hashimoto[1] | Natsuka Kimura[2] | Jun Narita[1] | Ryo Ishii[1] | Masaki Hirose[1] | Ken Hayashi[1] | Haruka Nishino[1] | Atsuko Ueyama[1] | Kazutoshi Ueda[1] | Chika Yoshihara[1] | Yasuji Kitabatake[1] | Ryozo Nagai[3] | Kenichi Aizawa[2] | Hidekazu Ishida[1]

[1]Department of Pediatrics, Osaka University Graduate School of Medicine, Osaka, Japan | [2]Division of Clinical Pharmacology, Department of Pharmacology, Jichi Medical University, Shimotsuke, Japan | [3]Jichi Medical University, Shimotsuke, Japan

Correspondence: Kenichi Aizawa (aizawa@jichi.ac.jp) | Hidekazu Ishida (hideishi@ped.med.osaka-u.ac.jp)

Received: 2 February 2025 | **Revised:** 25 March 2025 | **Accepted:** 17 April 2025

Funding: This study was supported by JSPS KAKENHI no. 23K07530 and the Miyata Foundation.

Keywords: heart transplantation | immunosuppressive treatment | liquid chromatography–tandem mass spectrometry | therapeutic drug monitoring

ABSTRACT

Background: Management of immunosuppressive therapy after heart transplantation is typically guided by blood trough levels. Although tissue concentrations of immunosuppressive agents reportedly correlate with acute rejection in liver and kidney allografts, data in pediatric heart transplant recipients remain limited.

Methods: We enrolled 41 pediatric heart transplant recipients who underwent follow-up endomyocardial biopsy (EMB) between July 2021 and December 2023. For nine of those patients, serial data were collected up to 24 weeks post-transplantation. Myocardial tissue concentrations of tacrolimus (TAC), everolimus (EVR), mycophenolic acid (MPA), and mycophenolic acid glucuronide (MPAG) were measured by liquid chromatography–tandem mass spectrometry (LC–MS/MS), while blood concentrations were quantified by LC–MS/MS or immunoassays.

Results: Significant correlations were observed between myocardial and blood concentrations at EMB for TAC ($r = 0.73$, $p < 0.0001$), MPA ($r = 0.79$, $p < 0.0001$), and MPAG ($r = 0.50$, $p < 0.0001$). However, for EVR there was no significant correlation. Longitudinal analysis demonstrated that the tissue-to-blood TAC and EVR ratios decreased with age. No significant rejection events were observed during the study period, precluding the analysis of rejection risk.

Conclusion: Myocardial TAC, MPA, and MPAG concentrations are associated with blood levels, whereas those of EVR showed no significant correlation. Further, the tissue perfusion efficiencies of TAC and EVR decreased with age. This study highlights the value of LC–MS/MS for immunosuppressant monitoring after pediatric heart transplantation.

1 | Introduction

Advances in immunosuppressive therapy have significantly improved the prognosis after heart transplantation due to their crucial role in mitigating the risk of rejection [1]. Subtherapeutic concentrations of immunosuppressive agents increase the risk of rejection, while supratherapeutic levels are associated with adverse outcomes, including opportunistic infections and nephrotoxicity [2]. Accordingly, given the substantial interindividual variability in drug metabolism and response, strict therapeutic

Abbreviations: CyA, cyclosporine A; eGFR, estimated glomerular filtration rate; EMB, endomyocardial biopsy; EVR, everolimus; IA, immunoassay; LC–MS/MS, liquid chromatography–tandem mass spectrometry; MPA, mycophenolic acid; MPAG, mycophenolic acid glucuronide; MS, mass spectrometry; TAC, tacrolimus.

drug monitoring is essential [3, 4]. Currently, immunosuppressive drug dosing is predominantly guided by trough blood concentrations, typically measured using immunoassays (IA) [3]. However, no robust correlations between trough levels and rejection have been established so far [1], as IA rely on antigen–antibody interactions, but do not directly quantify levels of the compound itself, potentially limiting their accuracy.

In contrast, recent advancements in mass spectrometry (MS) have allowed precise quantification of drug concentrations, even in small tissue samples. Unlike IAs, MS allows direct measurement of immunosuppressive agents. In kidney and liver transplant recipients, tissue concentrations of immunosuppressive agents often do not correlate with blood concentrations, with tissue levels possibly more predictive of graft outcomes [5–7]. Although a recent study in adult heart transplant recipients reported that low myocardial tissue concentrations of tacrolimus were associated with acute cellular rejection [8, 9], no studies have explored the tissue concentrations of other immunosuppressive agents, nor have these relationships been investigated in pediatric heart transplant recipients.

Thus, this study aimed to assess the myocardial tissue concentrations of immunosuppressive agents in pediatric heart transplant recipients and to examine the relationship between tissue and blood concentrations using MS. Additionally, we aimed to evaluate the utility of cardiac tissue drug monitoring for predicting the risk of acute cellular rejection in this patient population.

2 | Methods

2.1 | Study Population

This study focused on pediatric heart transplant recipients who underwent follow-up cardiac catheterization at Osaka University Hospital, Japan, between July 2021 and December 2023. Samples were obtained from 41 patients. Among them, serial sample collections were conducted for 9 patients. In total, 93 cardiac tissue samples and their corresponding blood samples were analyzed.

2.2 | Ethical Statement

Ethical approval for the study was obtained from the Osaka University Clinical Research Review Committee (approval no. 21157). The study adhered to the ethical principles set forth in the Declaration of Helsinki. Written informed consent was obtained from all patients and/or their legal guardians.

2.3 | Blood Sample Preparation

Trough blood concentrations of immunosuppressive agents, including tacrolimus (TAC), everolimus (EVR), mycophenolic acid (MPA), and cyclosporine A (CyA), were measured on the morning of scheduled catheterization using IA, as detailed in the following section. In addition, blood samples collected during endomyocardial biopsy (EMB) were processed for both IA and MS.

2.4 | Endomyocardial Biopsy

Scheduled EMBs were performed annually for all post-transplant patients. During the first 6 months after transplantation, EMBs were performed at 1, 2, 4, 8, 12, and 24 weeks post-transplant at our institution. Cardiac tissue samples were obtained from the interventricular septum of the right ventricle, with typical sample sizes of 1–2 mm. Multiple tissue samples were collected for assessing rejection. The rejection grade was evaluated by specialized pathologists according to the 1990 and 2004 criteria established by the International Society for Heart and Lung Transplantation (ISHLT) [10]. In addition, a separate tissue sample was cryopreserved in liquid nitrogen immediately after collection, and immunosuppressive agent concentrations were subsequently measured by MS.

2.5 | Chemicals and Reagents

The DOSIMYCO Calibrators (including MPA and MPAG) and DOSIMYCO Internal Standards (including $^{13}C_5$,$^{2}H_3$-MPA and ^{13}C,$^{2}H_3$-MPAG), as well as DOSIMMUNE Calibrators (including TAC, EVR, and CyA) and DOSIMMUNE Internal Standards (including ^{13}C,$^{2}H4$-TAC, $^{13}C_2$,$^{2}H_4$-EVR and $^{2}H_{12}$-CyA), along with the extraction buffer, were obtained from Shimadzu (Kyoto, Japan; Manufacturer: Alsachim, Illkirch-Graffenstaden, France). DOSIMYCO and DOSIMMUNE are reagent kits designed for immunosuppressive agent quantification. All other solvents were purchased from FUJIFILM Wako Pure Chemical (Osaka, Japan).

2.6 | Preparation of Blood Samples and Calibrators for all Immunosuppressive Agents

For MPA and MPAG measurements, 10 μL of plasma samples, 10 μL of the internal standard mix (including $^{13}C_5$,$^{2}H_3$-MPA and ^{13}C,$^{2}H_3$-MPAG), and 100 μL of the extraction buffer were combined and vortexed for 60 s, followed by centrifugation at 12000 rpm for 10 min at 4°C. Then, a 90 μL aliquot of the supernatant was transferred into vials for LC–MS/MS analysis, with an injection volume of 1 μL. For TAC, EVR, and CyA measurements, 20 μL of whole blood samples, 20 μL of the internal standard mix (including ^{13}C,$^{2}H_4$-TAC, $^{13}C_2$,$^{2}H_4$-EVR, and $^{2}H_{12}$-CyA), and 100 μL of extraction buffer were combined and vortexed for 60 s, followed by centrifugation at 12000 rpm for 10 min at 4°C. Then, a total of 80 μL of the supernatant was transferred into vials for LC–MS/MS analysis, with an injection volume of 40 μL.

2.7 | Preparation of Tissue Samples

Tissue samples weighing 0.8–3.6 mg were placed into 2 mL hard tubes and homogenized (6.5 m/s, 5 s, 3 times) by adding 30 μL of an internal standard mix (including $^{13}C_5$,$^{2}H_3$-MPA and ^{13}C,$^{2}H_3$-MPAG, ^{13}C,$^{2}H4$-TAC, $^{13}C_2$,$^{2}H_4$-EVR and $^{2}H_{12}$-CyA), 100 μL of extraction buffer, and 5-mm stainless steel beads. After centrifugation (15000 rpm, 10 min, 4°C), 80 μL of the supernatant was transferred into vials for LC–MS/MS analysis. For TAC, EVR, CyA, MPA, and MPAG quantification in tissue samples, the same tissue extracts were used but with different injection

volumes: 1 μL for TAC, EVR, and CyA, and 40 μL for MPA and MPAG; each measured using different methods.

To calculate the relative concentrations of immunosuppressive agents in the cardiac tissue to the blood, the [cardiac tissue concentration (ng/mg)] to [blood concentration at the time of EMB (μg/mL) measured by MS] ratio was used.

2.8 | LC–MS/MS Conditions

Immunosuppressive agent concentrations were analyzed using an LC–MS/MS system (LCMS-8050, Shimadzu, Kyoto, Japan). For liquid chromatography (LC) analyses, a DOSIMYCO Analytical column (Shimadzu) was utilized, with the column oven maintained at 50°C and the autosampler set to 4°C. The mobile phase A consisted of 90% 3 mM ammonium formate in water and 10% methanol, and mobile phase B contained 3 mM ammonium formate in methanol. The flow rate was set to 0.4 mL/min. The gradient program was as follows: 0–0.5 min, %B = 10; 0.5–2.5 min, %B = 10–75 gradients; 2.51–3.9 min, %B = 95; 4–5 min, %B = 10. TAC, EVR, CyA, MPA, and MPAG were detected in the electrospray ionization-positive mode. MS/MS conditions were as follows: nebulizer gas flow, 3 L/min; heating gas flow, 10 L/min; interface temperature, 300°C; desolvation line temperature, 526°C; heat block temperature, 400°C; and drying gas flow, 10 L/min. The collision energies and multiple reaction monitoring parameters for each analyte are provided in Table S1. Sample concentrations were quantified by calculating the area ratio relative to the internal standard reagent added to each sample. The accuracy and linearity of the calibration curves are shown in Table S2. Intra-day and interday variability are shown in Table S3. Intra-day and interday variability were determined using human plasma spiked with MPA and MPAG, whereas TAC, EVR, and CyA were spiked into human whole blood.

2.9 | Immunoassays

Blood concentrations of all immunosuppressive agents were measured using standard IA techniques commonly applied in clinical practice. TAC concentrations were determined using a chemiluminescent IA, while EVR levels were measured by latex agglutination turbidimetric IA, and MPA concentrations through enzyme-multiplied IA.

2.10 | Clinical Manifestations

The clinical characteristics of the patients were retrospectively obtained from their medical records. These data included age, sex, body weight, year of transplantation, history of rejection, types of immunosuppressive agents administered, and the dosage of immunosuppressive agents at the time of EMB.

2.11 | Statistical Analysis

We examined the associations between blood concentrations, myocardial tissue concentrations, and myocardial rejection grades. Statistical analyses were performed using the JMP Pro 17 software. Linear regression analysis was employed to assess correlations between groups, while the Steel–Dwass test was utilized to evaluate the relationship between myocardial pathology grades and drug concentrations. A $p < 0.01$ was considered statistically significant.

3 | Results

3.1 | Clinical Characteristics

The clinical profiles of the 41 pediatric heart transplant recipients are summarized in Table 1. Of these, 20 were male and 21 were female. The median age at the time of transplantation was 6.5 years (interquartile range [IQR]: 2–12.75), and the median age at the time of sampling was 12 years (IQR: 7–18). The median duration since transplantation was 3.5 years (IQR: 2–7).

The immunosuppressive regimens at the time of sampling are also shown in Table 1. Of the 41 patients, 25 (61%) were receiving TAC and mycophenolate mofetil (MMF); 8 patients (20%) were treated with TAC, MMF, and EVR; 4 patients (11%) with TAC and EVR; 3 patients (7%) received CyA, MMF, and EVR; and 1 patient (2%) received EVR and MMF. No deaths occurred during the observation period. CyA concentrations were excluded from further statistical analysis because of the small number of patients ($n = 3$) receiving it.

3.2 | Comparative IA and MS Analyses in Blood Samples

We first assessed the correlation between the drug concentrations in blood samples measured by IA and MS at EMB. Significant positive correlations were observed between IA and MS measurements for TAC ($r = 0.96$, $p < 0.0001$), EVR ($r = 0.83$, $p < 0.0001$), and MPA ($r = 0.98$, $p < 0.0001$; Figure 1A–C).

TABLE 1 | Patients' clinical characteristics.

	***N* = 41**
Male:Female	20:21
Age at transplantation (years old) (Median, IQR)	6.5 (2–12.75)
Age at study investigation (years old) (Median, IQR)	12 (7–18)
Years after transplantation (Median, IQR)	3.5 (2–7)
Immunosuppressants (*n*, %)	
TAC + MMF	25 (61%)
TAC + EVR + MMF	8 (20%)
TAC + EVR	4 (10%)
CyA + EVR + MMF	3 (7%)
EVR + MMF	1 (2%)

Abbreviations: CyA, cyclosporine A; EVR, everolimus; IQR, interquartile range; MMF, mycophenolate mofetil; TAC, tacrolimus.

3.3 | Correlation Between Blood and Cardiac Tissue Samples

Next, we evaluated the correlation between blood and cardiac tissue concentrations for each immunosuppressive agent. Three types of blood concentrations were analyzed: trough concentrations measured by IA, blood concentrations at EMB measured by IA, and blood concentrations at EMB measured by MS.

For TAC, blood concentrations significantly correlated with cardiac tissue concentrations: trough levels ($r=0.59$, $p<0.0001$), levels at EMB measured by IA ($r=0.74$, $p<0.0001$), and levels at EMB measured by MS ($r=0.73$, $p<0.0001$; Figure 2A–C). Notably, blood samples obtained at EMB exhibited stronger correlations with cardiac tissue concentrations compared with trough levels.

In contrast, EVR concentrations in blood samples did not demonstrate significant correlations with cardiac tissue concentrations: trough levels ($r=0.052$, $p=0.82$), levels at EMB measured by IA ($r=0.40$, $p=0.075$), and levels at EMB measured by MS ($r=0.19$, $p=0.39$; Figure 2D–F).

For MPA, trough concentrations did not significantly correlate with cardiac tissue concentrations ($r=0.18$, $p=0.13$). However, MPA concentrations measured by IA and MS at EMB significantly correlated with cardiac tissue concentrations: EMB by IA ($r=0.75$, $p<0.0001$) and EMB by MS ($r=0.79$, $p<0.0001$; Figure 2G–I). These findings may be attributed to the metabolism of MPA, which exhibits enterohepatic circulation [11, 12]. Additionally, the MPAG concentration measured at EMB by MS was significantly correlated with cardiac tissue MPAG concentrations ($r=0.50$, $p<0.0001$; Figure 2J).

3.4 | Correlations Between Age at Sampling and Years After Transplantation and Cardiac Tissue Concentration

Given that our study cohort comprised pediatric patients, we analyzed the correlation between cardiac tissue concentrations and the age at the time of sampling. Additionally, we evaluated the relationships between cardiac tissue concentrations and the time after transplantation. As target doses of immunosuppressive agents typically decrease with time after transplantation, we calculated relative cardiac tissue-to-blood ratios measured by MS at the time of EMB.

Intriguingly, the relative cardiac tissue concentration of TAC ($r=-0.50$, $p<0.0001$) and EVR ($r=-0.56$, $p=0.0086$) demonstrated a significant inverse correlation with age at the time of EMB (Figure 3A,B). In contrast, those of MPA ($r=-0.098$, $p=0.39$) and MPAG ($r=-0.19$, $p=0.09$) did not exhibit any significant correlation with age at EMB (Figure 3C,D). With respect to the time since heart transplantation, the relative TAC concentration showed no significant correlation with time since transplantation ($r=-0.07$, $p=0.040$; Figure 3E), nor did the concentrations of EVR ($p=0.38$), MPA ($p=0.82$), and MPAG ($p=0.82$; Figure 3F–H).

3.5 | Correlation Between Immunosuppressive Agent Dosage and Blood or Tissue Concentration

Next, we analyzed the correlations between the dosage of immunosuppressive agents and their corresponding blood or tissue concentrations. The dosage was standardized based on body weight, calculated as dosage per day (mg/day) divided by body weight (kg). Then, we compared the standardized dosages (mg/kg/day) with blood and cardiac tissue concentrations.

TAC concentrations, including trough levels, blood levels at sampling, and cardiac tissue levels, were significantly correlated with TAC dosage (Figure 4A–C). Notably, the strongest correlation coefficient was observed for the cardiac tissue concentration. In contrast, EVR concentration at trough levels and in blood at sampling was not significantly correlated with EVR dosage. However, the cardiac tissue concentrations of EVR showed a significant correlation with EVR dosage (Figure 4D–F). Regarding MMF, its dosage was significantly correlated with MPA blood concentrations at trough levels and sampling time points, whereas no significant correlation was observed between MMF dosage and cardiac tissue MPA concentration (Figure 4G–I). Concerning MPAG, blood concentration at sampling was significantly correlated with MMF dosage, whereas cardiac tissue MPAG concentration showed no correlation with MMF dosage (Figure 4J,K).

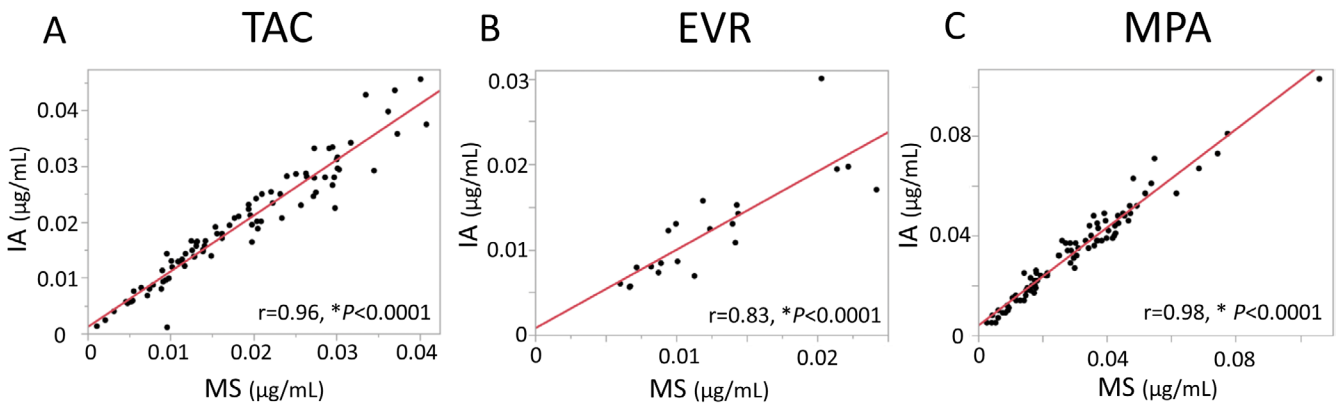

FIGURE 1 | Correlation of drug concentrations between immunoassays (IA) and mass spectrometry (MS). All blood samples obtained at the time of cardiac biopsy were analyzed for tacrolimus (TAC; A; $n=86$), everolimus (EVR; B; $n=21$), and mycophenolic acid (MPA; C; $n=87$).

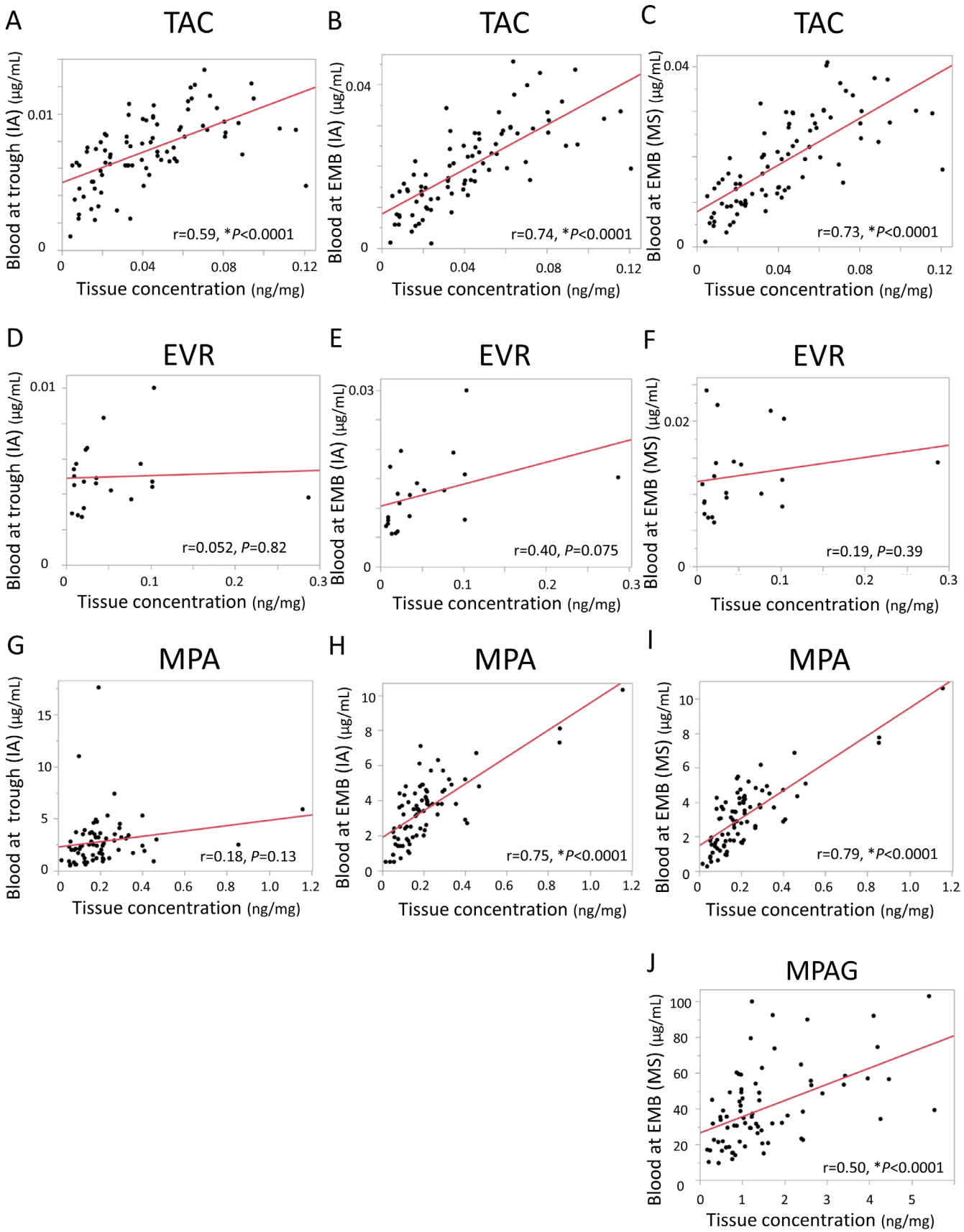

FIGURE 2 | Correlation between drug concentrations in blood and cardiac tissue. Blood concentrations for tacrolimus (TAC; A–C; $n = 84$), everolimus (EVR; D–F; $n = 21$), mycophenolic acid (MPA; G–I; $n = 79$), and mycophenolic acid glucuronide (MPAG; J; $n = 76$) were measured at the trough by immunoassay (IA) and at the time of endomyocardial biopsy (EMB) by IA and mass spectrometry (MS). All cardiac tissue concentrations were measured by MS.

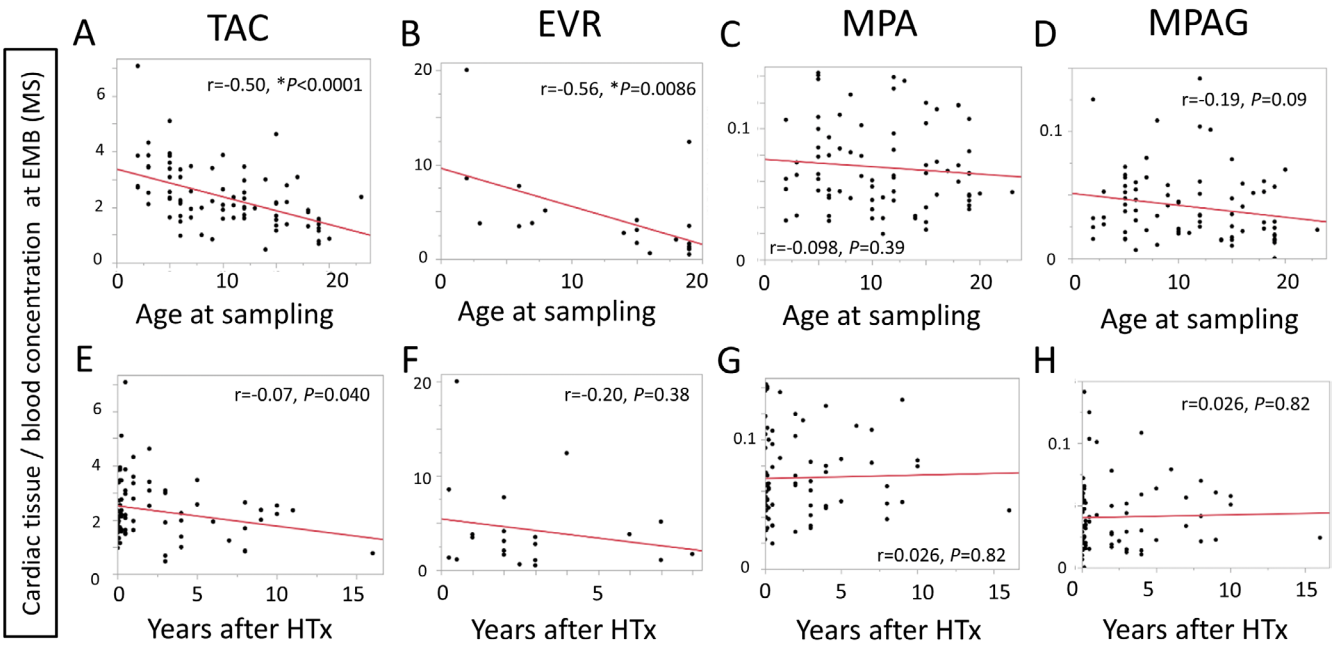

FIGURE 3 | Correlations between the ratio of cardiac tissue and blood drug concentrations at endomyocardial biopsy (EMB) toward the age at sampling and the time after heart transplantation. All blood and cardiac tissue samples were analyzed by mass spectrometry (MS). Cardiac tissue-to-blood ratios were calculated for tacrolimus (TAC; A and E; $n = 84$), everolimus (EVR; B and F; $n = 21$), mycophenolic acid (MPA; C and G; $n = 80$), and mycophenolic acid glucuronide (MPAG; D and H; $n = 77$), and compared with age at sampling (A–D) and years after heart transplantation (HTx; E–H).

3.6 | Correlation Between Cardiac Tissue Concentration and Acute Cellular Rejection

To investigate whether the cardiac tissue concentrations of immunosuppressive agents correlate with the risk of acute cellular rejection in pediatric cardiac allografts, we examined the relationship between these concentrations and the ISHLT grading of acute cellular rejection. In our study cohort, there were no instances of significant cellular rejection above Grade 3 according to ISHLT 1990 criteria (or Grade 2R according to ISHLT 2004 criteria).

Among rejection grades 0, 1A, 1B, and 2, we observed no significant differences in cardiac tissue concentrations of TAC, EVR, MPA, or MPAG (Figure 5A–D) and the cardiac tissue-to-blood ratio at the time of EMB (Figure S1A–D). As a result, we could not establish a definitive association between cardiac tissue concentrations of immunosuppressive agents and significant acute cellular rejection in pediatric cardiac allografts.

3.7 | Time Profile of Immunosuppressive Agent Concentrations in Cardiac Tissue

Finally, we analyzed sequential time profiles of immunosuppressive agent concentrations in the cardiac tissue of each patient after heart transplantation. During the study period, nine patients underwent heart transplantation. Given the varying target therapeutic range of TAC trough levels at different time points post-transplantation, we focused on cardiac tissue-to-blood ratios. Relative TAC concentrations were relatively lower around 4 weeks post-transplantation (Figure 6A), but not significantly. In contrast, no such trend was observed for MPA concentrations (Figure 6B). On the other hand, EVR analysis was not feasible because a limited number of patients received EVR within the first 24 weeks post-transplantation.

4 | Discussion

This study is the first to quantify cardiac tissue concentrations of immunosuppressive agents in pediatric heart transplant recipients using MS. Our findings indicate that the blood concentrations of TAC, EVR, and MPA measured by IA correlate well with those obtained via MS. In clinical practice, blood immunosuppressive agent concentrations are typically monitored using IA. This study confirmed that blood TAC, EVR, and MPA concentrations measured by IA correlated with those measured by MS even in pediatric patients.

Previous studies in liver and kidney allografts demonstrated that TAC tissue concentrations correlate with acute rejection and are not always reflective of blood levels [5–7]. A previous study on adult heart transplant recipients indicated that patients with acute cellular rejection had relatively lower TAC levels in the cardiac tissues during the first year post-transplant [8]. However, due to its sample size, this study could not demonstrate a significant correlation between TAC tissue concentrations and acute rejection. In the present study, we demonstrated a significant correlation between TAC blood and cardiac tissue concentrations. In contrast, the EVR concentrations in the blood and cardiac tissue were not significantly correlated. To the best of our knowledge, no prior study examined EVR concentrations in allograft tissue. While the reason for the discrepancy between TAC and EVR remains unclear, EVR may have unique tissue perfusion characteristics. Conversely, MPA and its metabolite MPAG showed significant correlations between

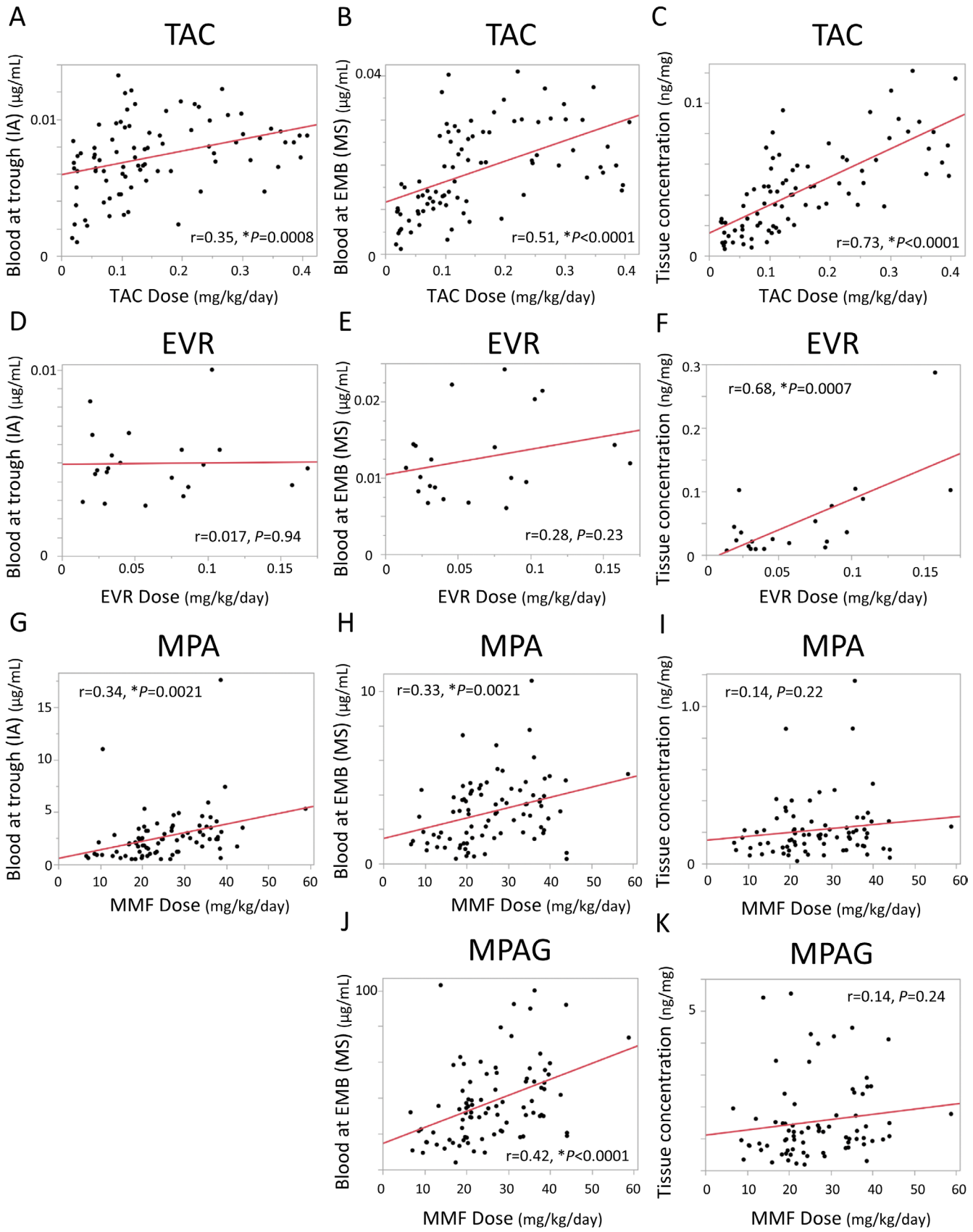

FIGURE 4 | Correlation between drug dosage and blood or cardiac tissue concentrations. Blood and tissue concentrations for tacrolimus (TAC; A–C; $n = 84$), everolimus (EVR; D–F; $n = 21$), mycophenolic acid (MPA; G–I; $n = 79$), and mycophenolic acid glucuronide (MPAG; J and K; $n = 76$) are compared with drug dosages at sampling.

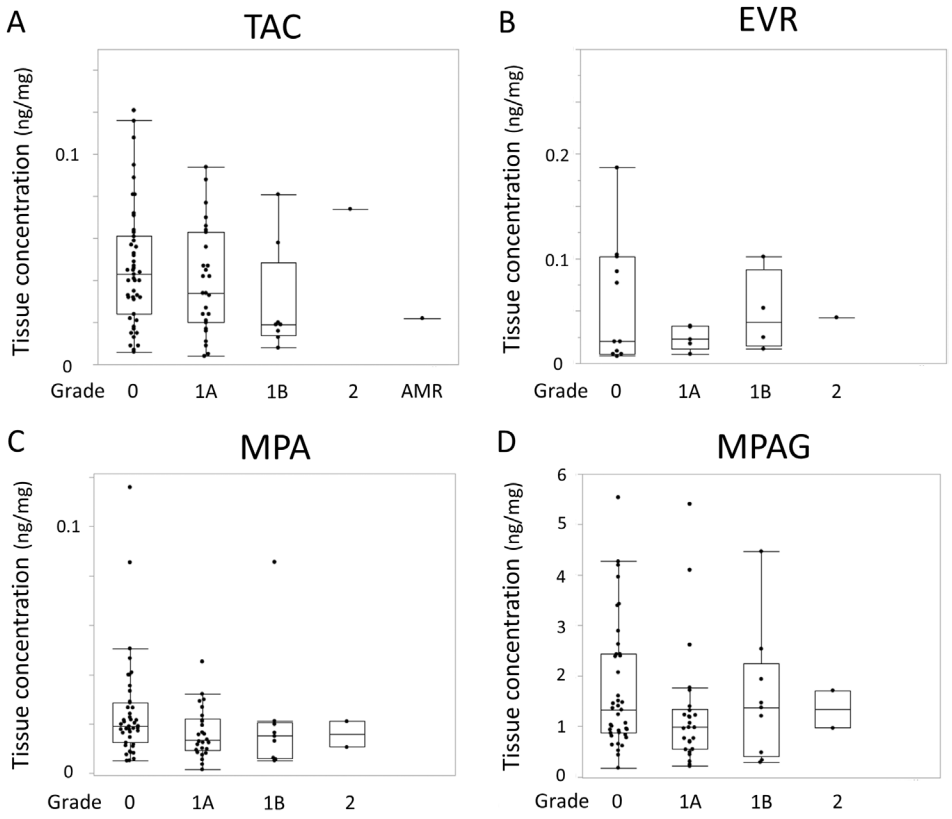

FIGURE 5 | Comparison of cardiac tissue concentrations of TAC (A), EVR (B), MPA (C), MPAG (D), and acute cellular rejection grade. The grade of acute cellular rejection was classified as 0 ($n = 50$), 1A ($n = 30$), 1B ($n = 10$), and 2 ($n = 2$) according to the criteria of the International Society of Heart and Lung Transplantation 1990. One patient was diagnosed with antibody-mediated rejection (AMR). No patient showed ≥ Grade 3. No significant differences were observed among groups in the cardiac tissue concentration of each immunosuppressant.

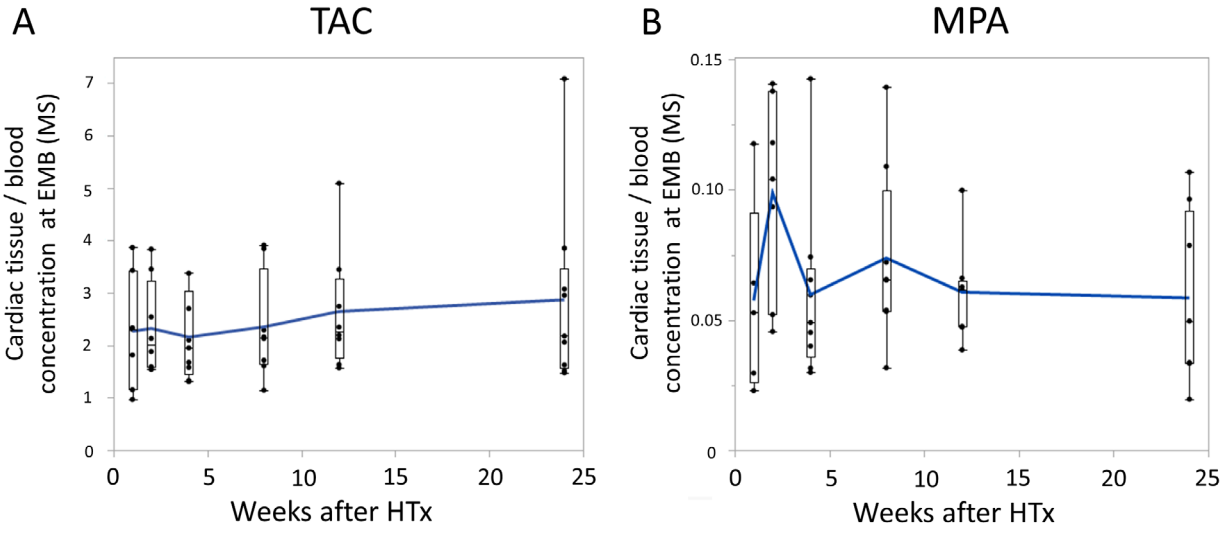

FIGURE 6 | Sequential analysis of cardiac tissue-to-blood ratios of TAC (A) and MPA (B) at endomyocardial biopsy (EMB). Nine patients who received heart transplantation during the study period were analyzed. The cardiac tissue-to-blood ratios at EMB for tacrolimus (TAC) and mycophenolic acid (MPA) are shown according to weeks after heart transplantation (HTx). Line chart shows the mean values of the samples. There were no significant differences among time points.

blood concentrations at the time of EMB and cardiac tissue concentrations, although MPA trough levels did not correlate with tissue concentrations. This is likely due to MPA's unique pharmacokinetics, particularly its enterohepatic circulation [11, 12]. Notably, we observed significantly decreased cardiac tissue-to-blood TAC and EVR concentration according to the patients' age, indicating that the tissue perfusion efficiencies of TAC and EVR were affected by age. This pattern was observed for TAC and EVR but not for MPA and MPAG. Accordingly, we may reduce TAC and EVR dosages in younger recipients.

Additionally, the correlations between dosages and cardiac tissue concentrations varied among immunosuppressive drugs. These results may reflect the pharmacokinetic properties of each agent; TAC and EVR are highly lipophilic and accumulate in cardiac tissue with long-term dosing, whereas MPA and MPAG are more dependent on plasma exposure because of enterohepatic recirculation and limited tissue permeability. However, the precise mechanisms underlying these findings could not be determined in this study. Further research on pharmacokinetics is needed to clarify this point.

In addition, we aimed to assess the relationship between the cardiac tissue concentrations of immunosuppressive agents and acute cellular rejection; however, no patient in our cohort experienced significant acute rejection (≥ Grade 3 according to ISHLT 1990 or Grade 2R according to ISHLT 2004) during the study period. Consequently, we could not determine whether the cardiac allograft immunosuppressive agent concentrations were associated with rejection risk. A previous study in adult heart transplant patients showed lower TAC concentrations in cardiac biopsy samples of patients with acute rejection, although the authors were unable to perform statistical analyses due to the small sample size [8]. Further studies with larger cohorts are needed to elucidate the relationship between cardiac tissue concentrations of immunosuppressive agents and the risk of acute cellular rejection.

Finally, we analyzed cardiac tissue concentrations of immunosuppressive agents during the first 6 months post-transplantation in nine pediatric patients. Although no significant trends were observed, there was a tendency for relative cardiac tissue TAC to blood levels to be lower around 4 weeks post-transplant. A similar trend was reported in a previous study of adult heart transplant patients, where the relative cardiac TAC concentrations at 3 months were lower than at other time points [8]. This variation in cardiac tissue drug perfusion may be linked to an increased risk of rejection during the first 1–3 months post-transplantation.

This study has several limitations. First, the sample size was relatively small, limiting the strength of our conclusions. Notably, none of the patients required additional immunosuppressive treatment for rejection. Thus, we could not determine the predictive value of the tissue concentrations in cardiac allografts. Further longitudinal studies are needed for investigating the correlation between immunosuppressive agent concentrations in cardiac allografts and the rejection risk.

5 | Conclusion

After pediatric heart transplantation, the cardiac tissue concentrations of TAC, MPA, and MPAG significantly correlated with their corresponding blood concentrations at the time of sampling. In contrast, EVR concentrations did not demonstrate a correlation with blood levels. The TAC and EVR tissue perfusion efficiencies decreased with patients' ages. The relationship between acute cellular rejection and cardiac tissue concentrations could not be elucidated in this study because no patients experienced significant acute rejection during the observation period.

Acknowledgments

This study was supported by JSPS KAKENHI no. 23K07530 and the Miyata Foundation.

Ethics Statement

Ethical approval for the study was obtained from the Osaka University Clinical Research Review Committee (approval no. 21157).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. A. Velleca, M. A. Shullo, K. Dhital, et al., "The International Society for Heart and Lung Transplantation (ISHLT) Guidelines for the Care of Heart Transplant Recipients," *Journal of Heart and Lung Transplantation* 42, no. 5 (2023): e1–e141.

2. M. A. Sikma, E. M. van Maarseveen, E. A. Graaf, et al., "Pharmacokinetics and Toxicity of Tacrolimus Early After Heart and Lung Transplantation," *American Journal of Transplantation* 15, no. 9 (2015): 2301–2313, https://doi.org/10.1111/ajt.13309.

3. M. Brunet, T. van Gelder, A. Åsberg, et al., "Therapeutic Drug Monitoring of Tacrolimus-Personalized Therapy: Second Consensus Report," *Therapeutic Drug Monitoring* 41, no. 3 (2019): 261–307, https://doi.org/10.1097/FTD.0000000000000640.

4. I. Gueta, N. Markovits, H. Yarden Bilavsky, et al., "High Tacrolimus Trough Level Variability Is Associated With Rejections After Heart Transplant," *American Journal of Transplantation* 18, no. 10 (2018): 2571–2578.

5. W. J. Sandborn, G. M. Lawson, T. J. Cody, et al., "Early Cellular Rejection After Orthotopic Liver Transplantation Correlates With Low Concentrations of FK506 in Hepatic Tissue," *Hepatology* 21, no. 1 (1995): 70–76, https://doi.org/10.1002/hep.1840210113.

6. A. Capron, J. Lerut, C. Verbaandert, et al., "Validation of a Liquid Chromatography-Mass Spectrometric Assay for Tacrolimus in Liver Biopsies After Hepatic Transplantation: Correlation With Histopathologic Staging of Rejection," *Therapeutic Drug Monitoring* 29, no. 3 (2007): 340–348, https://doi.org/10.1097/FTD.0b013e31805c73f1.

7. M. Bodnar Broniarczyk, M. Durlik, T. Bączkowska, et al., "Kidney and Liver Tissue Tacrolimus Concentrations in Adult Transplant Recipients—The Influence of the Whole Blood and Tissue Concentrations on Efficiency of Treatment During Immunosuppressive Therapy," *Pharmaceutics* 13, no. 10 (2021): 1576, https://doi.org/10.3390/pharmaceutics13101576.

8. S. De Gregori, A. De Silvestri, B. Cattadori, et al., "Therapeutic Drug Monitoring of Tacrolimus-Personalized Therapy in Heart Transplantation: New Strategies and Preliminary Results in Endomyocardial Biopsies," *Pharmaceutics* 14, no. 6 (2022): 1247.

9. M. Molinaro, C. Pellegrini, B. Cattadori, and S. de Gregori, "Development and Validation of a Combined Enzymatic-Digestion/Mass Spectrometry Assay for Tacrolimus Quantitation in Cardiac Biopsies," *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences* 1152 (2020): 122215.

10. S. Stewart, G. L. Winters, M. C. Fishbein, et al., "Revision of the 1990 Working Formulation for the Standardization of Nomenclature in the

Diagnosis of Heart Rejection," *Journal of Heart and Lung Transplantation* 14, no. 11 (2005): 1710–1720.

11. R. E. Bullingham, A. J. Nicholls, and B. R. Kamm, "Clinical Pharmacokinetics of Mycophenolate Mofetil," *Clinical Pharmacokinetics* 34, no. 6 (1998): 429–455.

12. L. T. Weber, M. Shipkova, V. W. Armstrong, et al., "The Pharmacokinetic-Pharmacodynamic Relationship for Total and Free Mycophenolic Acid in Pediatric Renal Transplant Recipients: A Report of the German Study Group on Mycophenolate Mofetil Therapy," *Journal of the American Society of Nephrology* 13, no. 3 (2002): 759–768, https://doi.org/10.1681/ASN.V133759.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.