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Short communication

Simultaneous determination of mycophenolic acid and its glucuronides in human plasma using isocratic ion pair high-performance liquid chromatography

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Abstract

Therapeutic drug monitoring (TDM) of mycophenolic acid (MPA) following administration of mycophenolate mofetil (MMF) or the enteric-coated sodium salt of MPA formulations, seems beneficial because of the large intra- and inter-individual variability in MPA pharmacokinetics. MPA is an active component from these oral formulations and are further metabolised to inactive phenolic glucuronide (MPAG) and active acyl glucuronide (AcMPAG). This study aims to determine simultaneously these three metabolites of MMF using isocratic ion pair HPLC and to evaluate the short-term stability of AcMPAG in human plasma. Samples were prepared using solid phase extraction. Chromatographic separation was achieved over an RP column (TSKgel® ODS-80Ts, 150 mm × 4.6 mm i.d., 5 μm particle size) with acetonitrile and 30 mM tetra-*n*-butylammonium bromide containing 5 mM ammonium acetate at pH 9.0 (33/67, v/v) as the mobile phase. The flow rate of the mobile phase was 1 ml/min, and the wavelength of determination by UV detection was 250 nm (run time, 16 min). Calibration curves for MPA, MPAG and AcMPAG in human plasma were linear over a concentration range of 0.05–50, 0.1–400 and 0.08–8 μg/ml, respectively. Intra- and inter-assay R.S.D. were < 6.5%. Extraction efficiencies were more than 85% for all analytes. Since AcMPAG was unstable in human plasma, plasma acidification was needed for the quantification of AcMPAG. Large interindividual variability was observed in the AcMPAG pharmacokinetics in the early period after renal transplantation. In conclusion, a simple, accurate and reproducible HPLC method to measure simultaneously these three MMF metabolites has been established. The method will be helpful in evaluating pharmacokinetics of MPA and its glucuronides.
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Keywords: Mycophenolic acid; Mycophenolic acid acyl glucuronide; Mycophenolic acid phenolic glucuronide; Human plasma concentration; Isocratic ion pair HPLC

1. Introduction

The pro-drug mycophenolate mofetil (MMF) is an immunosuppressant with proven efficacy in the prophylaxis of rejection in organ transplantation [1–3]. Mycophenolic acid (MPA), an active derivative of MMF, can inhibit selectively and reversibly the rate-limiting enzyme inosine monophosphate dehydrogenase type 2 (IMPDH-2) in *de novo* purine biosynthesis of guanine nucleotides in lymphocytes [4]. MPA is primarily glucuronidated to the major inactive MPA phenolic glucuronide metabolite (MPAG) and the minor active MPA acyl glucuronide metabolite (AcMPAG) by uridine diphosphate glucuronosyltransferase (UGT) at the phenolic hydroxyl group and the acyl group, respectively [5]. Although MPAG is pharmacologically inactive [6], it can be hydrolyzed back to MPA during enterohepatic recirculation. The contribution of enterohepatic recirculation to the total MPA exposure is approximately 40% in healthy subjects [7]. MPAG has been reported to displace MPA from its albumin binding site and increase its free fraction [8]. AcMPAG, another glucuronide of MPA, also has an inhibitory effect on IMPDH-2 and induces cytokine release [9–11]. AcMPAG seems to be associated with the gastrointestinal toxicity

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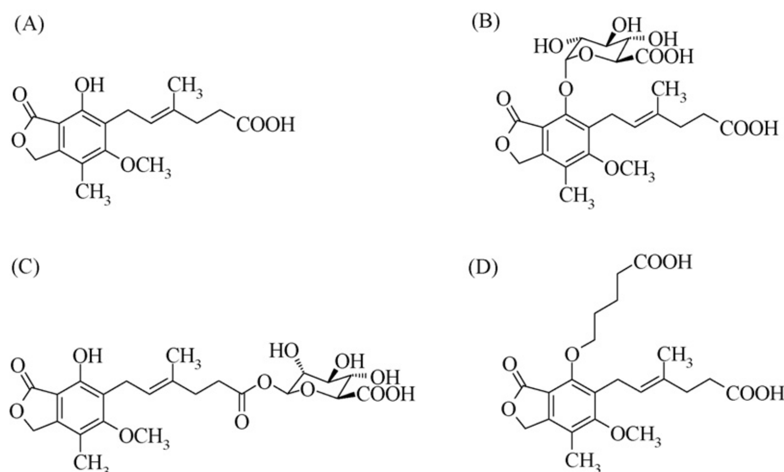

Fig. 1. Chemical structures of MPA (A), MPAG (B), AcMPAG (C) and MPAC (D).

of MPA [12]. However, it is controversial whether AcMPAG monitoring is helpful for predicting efficacy and toxicity in MPA therapy, because there is only one systematic study that assessed the association between AcMPAG plasma concentration and the incidence of diarrhea in patients on MMF [13].

Therapeutic drug monitoring (TDM) of MPA has been accepted as an essential tool in the management of immunosuppressive therapy in solid organ transplant recipients [1,14,15]. In general, enzyme immunoassay including enzyme multiplied immunoassay technique (EMIT) and cloned enzyme donor immunoassay (CEDIA) have the advantage of being less laborious and time-consuming than chromatographic techniques, and then suitable for TDM. However, cross-reactivity to AcMPAG has been observed in EMIT and CEDIA, because the antigenic determinant of the antibody to MPA is the phenolic hydroxy group of MPA or AcMPAG [16–18]. For instance, Schutz et al. reported that a substantial overestimation of 30–35% by the EMIT compared to HPLC was observed in adult liver and both adult and pediatric kidney transplant recipients [19]. Westley et al. showed a mean overestimation of 54% with the CEDIA method across transplant types [17]. TDM of MPA must be able to distinguish the relative contributions of MPA and AcMPAG to the reported test result.

Some groups reported gradient methods to determine plasma MPA, MPAG and AcMPAG in a single chromatographic run [20,21]. An isocratic HPLC method is simpler than gradient methods. Although the former is more useful in clinical practice, there are few reports with an isocratic HPLC method to determine MPA, MPAG and AcMPAG simultaneously in human plasma [22,23]. With respect to stability, it has been reported that AcMPAG was unstable, and plasma should be acidified in order to prevent the hydrolysis of AcMPAG [20,24]. However, it has been reported that AcMPAG was stable in human plasma [21,22]. The stability of AcMPAG in human plasma remains to be clarified.

This study aims to establish a simultaneous determination method for MPA, MPAG and AcMPAG using isocratic ion pair (IP) HPLC in human plasma in order to evaluate the short-term stability of AcMPAG, and to quantify these three metabolites of MMF in clinical samples obtained from renal transplant recipients.

2. Experimental

2.1. Chemicals

MPA was purchased from Sigma (St. Louis, MO, USA) and MPAG from Analytical Services International (London, UK). AcMPAG and a carboxy butoxy ether derivative of MPA (MPAC) were obtained from Roche Pharmaceuticals (Palo Alto, CA, USA) (Fig. 1). HPLC-grade acetonitrile and tetra-*n*-butylammonium bromide were purchased from Wako Pure Chemicals (Osaka, Japan). All other reagents were reagent grade and commercially available.

2.2. Sample extraction

Blood specimens were drawn into tubes containing EDTA and immediately stored at 4 °C until sample preparation. Plasma was obtained by centrifugation of the blood samples at 1670 × *g* at 4 °C for 10 min. One ml of the plasma was acidified with 25 μl of 10% acetic acid and then stored at −35 °C until analysis. The frozen plasma samples were thawed at room temperature. To 500 μl of the acidified plasma sample, 1 ml of distilled water and 50 μl of 50% acetonitrile containing MPAC (100 μg/ml) were added. This was then loaded onto a solid phase extraction (SPE) cartridge (Sep-Pak® plus C18, 360 mg, 0.7 ml, Waters, Milford, MA, USA) previously conditioned with 1.5 ml of acetonitrile, 3 ml of methanol and 3 ml of distilled water. The cartridge was washed with 500 μl of distilled water. The analytes were then eluted with 1.5 ml of 90% acetonitrile containing 0.25% ammonia solution, and 25 μl of 10% acetic acid immediately added to the eluent to prevent the alkaline hydrolysis of AcMPAG. The extract was evaporated to dryness. The residue was reconstituted in 120 μl of mobile phase and then injected into the analytic column.

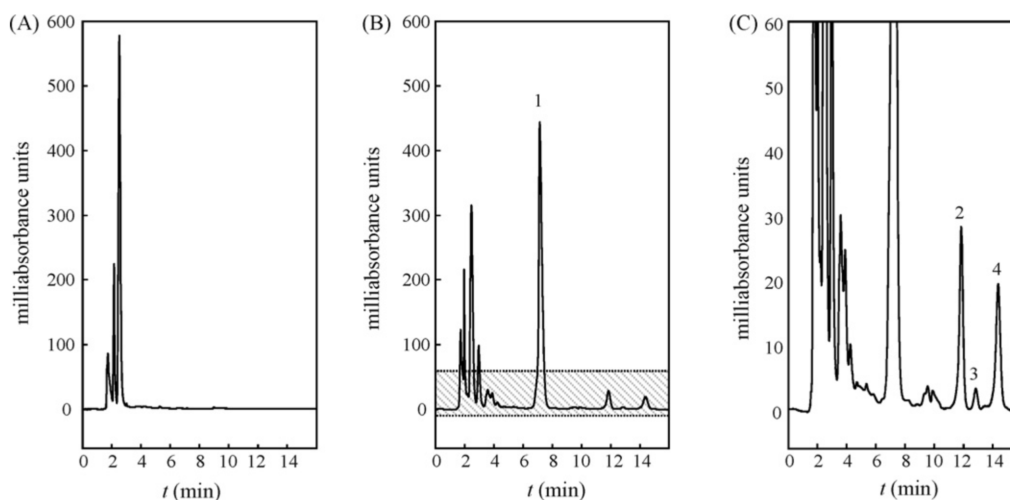

Fig. 2. Chromatograms of a drug-free extracted plasma sample (A) and a patient plasma sample receiving MMF (B and C): MPA, 4.29 μg/ml; MPAG, 86.0 μg/ml and AcMPAG, 0.858 μg/ml. Chromatogram of (C) has been enlarged to 10 times that of (B) in the striped area. Retention times are 11.9, 7.2, 12.9 and 14.4 min for MPA (2), MPAG (1), AcMPAG (3) and MPAC (4), respectively.

2.3. HPLC apparatus and conditions

The HPLC system (Shimadzu, Kyoto, Japan) consisted of a LC-10AT pump, SIL-10AXL autoinjector and SPD-M10AVP diode array detector. Data were collected and analyzed by Class-VP software Version 6.14 (Shimadzu). The separation was carried out on an octadecyl silane (ODS) column (TSKgel® ODS-80Ts, 150 mm × 4.6 mm i.d., 5 μm particle size, Tosoh, Tokyo, Japan). The mobile phase was a mixture of 30 mM tetra-*n*-butylammonium bromide containing 5 mM ammonium acetate pH 9.0 (adjusted with 1 M ammonia) and acetonitrile (67/33, v/v). The flow rate was 1 ml/min, the column temperature was 40 °C, and the autosampler was set at 4 °C. The wavelength of the photodiode array UV detector was set at 250 nm. The injection volume was 30 μl.

2.4. Method validation

Linear regression was calculated using the ratio of the peak areas for MPA, MPAG and AcMPAG to MPAC. Seven in-house QC standards were prepared in drug-free plasma with a final concentrations of 0.05, 0.5, 1.5, 4, 10, 20 and 50 μg/ml MPA, 0.1, 1, 20, 40, 90, 180 and 400 μg/ml MPAG and 0.08, 0.25, 0.5, 1, 2, 4 and 8 μg/ml AcMPAG. Accuracy was determined by evaluating the analytical recovery of known amounts of standard solutions added to plasma. The extraction efficiency was calculated by comparing peak areas obtained from the extracted plasma samples spiked with MPA, MPAG and AcMPAG with those obtained from mobile phase containing the same amount of these compounds directly injected onto the column without extraction. Intra- and inter-assay precisions were evaluated with control plasma spiked with four different concentrations of the analytes. The intra- and inter-assay precisions were expressed as R.S.D. (%).

2.5. Stability of AcMPAG in human plasma

The stabilities of AcMPAG were evaluated in fresh plasma samples obtained from healthy subjects. Samples were spiked with 4 μg/ml of AcMPAG. Acidified or nonacidified samples were stored separately at 20 or 4 °C and analyzed immediately after preparation of the pools and after 2, 4, 8 and 24 h of storage.

2.6. Ethics and blood sampling

The study was performed in accordance with the Declaration of Helsinki and its amendments. The protocol was approved by the Ethics Committee of Hamamatsu University Hospital. Each patient received information about the scientific aim of this study and gave their written informed consent. EDTA blood samples over a period of 12 h after morning drug administration were obtained from 6 renal transplant recipients 10 days after renal transplantation. These patients were treated with MMF, tacrolimus and prednisolone or methylprednisolone for immune suppression.

3. Results

3.1. Chromatographic separation

Chromatograms of a drug-free extracted plasma (A) and a plasma specimen from a patient receiving MMF (B and C) are shown in Fig. 2. MPA, MPAG, AcMPAG and MPAC were eluted at 11.9, 7.2, 12.9 and 14.4 min, respectively. The selectivity of the assay was checked by injecting blank plasma and referring to photodiode array spectrum. No peaks interfering with an analyte or the internal standard were observed.

3.2. Calibration curves

Calibration curves in human plasma were linear over a concentration range of 0.05–50 μg/ml for MPA, 0.1–400 μg/ml for

Table 1
Assay linearity and LOQ for MPA, MPAG and AcMPAG

Sample analytes	Linearity (μg/ml)	LOQ (μg/ml)	r
MPA	0.05–50.0	0.05	0.9997
MPAG	0.10–400	0.10	0.9996
AcMPAG	0.08–8.00	0.08	0.9997

MPA, Mycophenolic acid; MPAG, mycophenolic acid phenolic glucuronide; AcMPAG, mycophenolic acid acyl glucuronide.

MPAG and 0.08–8 μg/ml for AcMPAG (Table 1). The correlation coefficient was greater than 0.999 in all calibration curves ($n = 7$). The lower LOQ, defined as the concentration at which the R.S.D. does not exceed 20% ($n = 8$) were 50, 100 and 80 ng/ml for MPA, MPAG and AcMPAG, respectively.

3.3. Extraction efficiencies

The extraction efficiencies were 89.1, 97.5, 101.9 and 86.8% for MPA, MPAG, AcMPAG and MPAC, respectively ($n = 3$). Acidification of plasma samples before extraction improved recovery of AcMPAG (data not shown). Immediate acidification of eluent after extraction also increased extraction efficiency of AcMPAG by 9.2%. When eluted with only acetonitrile, the extraction efficiencies decreased to 71.4, 81.2, 75.1 and 41.8% for MPA, MPAG, AcMPAG and MPAC, respectively.

3.4. Assay accuracy and precision

The intra- and inter-assay precisions of MPA, MPAG and AcMPAG were 0.11–3.89 and 0.32–6.45, 0.48–5.34 and 0.40–2.92 and, 0.42–3.82 and 0.71–6.07%, respectively (Table 2).

3.5. Stability of AcMPAG in human plasma samples and mobile phase

AcMPAG was stable in acidified plasma specimens (pH < 6.5) at 4 °C up to 24 h. In nonacidified plasma specimens (pH 7.4) at 4 °C, 95% of the initial amount of AcMPAG remained after 8 h. AcMPAG decreased to 68% of the initial amount after 8 h in nonacidified plasma specimens at 20 °C (Fig. 3). AcMPAG was also stable over 24 h in mobile phase of pH 9.0 at 4 °C (data not shown).

3.6. Concentration–time profiles and incidence of diarrhea in renal transplant recipients

Fig. 4 shows the concentration–time profiles in 6 renal transplant recipients 10 days after transplantation on MMF therapy over a period of 12 h after drug administration. Interindividual variability was observed in plasma concentration of AcMPAG as well as MPA and MPAG concentration. Diarrhea was not observed in the 6 recipients.

4. Discussion

All peaks of the analytes and internal standard separated well from each other in human plasma samples, because mobile phase used was buffered with ammonium acetate (Fig. 2). No interfering peaks from co-administrated drugs and plasma extracts were observed in determination of MPA, MPAG and AcMPAG in 6 patients plasma samples who were receiving tacrolimus, prednisolone, methylprednisolone, amlodipine, doxazosin, metoprolol, furosemide, rabeprazole, amphotericin B or levofloxacin. Table 2 indicates that this method provides acceptable precision and accuracy in accordance with FDA guidelines [25]. With our sample purification, more than 800 chromatographic runs could be achieved with one conventional ODS column without any deterioration of the separation performance.

The possibility of eluting with nonalkaline conditions in SPE was initially tested in this study, because AcMPAG was reported to be unstable in alkaline conditions [5]. However, sufficient elution of all analytes in 1.5 ml of acetonitrile was not achieved. With alkaline conditions, higher extraction efficiencies of all analytes were obtained. However, the extraction recovery of AcMPAG decreased without immediate acidification of eluent after extraction. AcMPAG was unstable in alkaline condition as previously reported.

In this study, AcMPAG was unstable in nonacidified plasma specimens at 20 °C (Fig. 3). In acidified plasma specimens at 4 °C, AcMPAG was stable up to 24 h. This finding was similar to the results of Shipkova et al. [20] and Brandhorst et al. [24]. We acidified plasma samples with common acetic acid, while they used perchloric acid. Because acidification of extraction eluent also made AcMPAG stable, the acidification of plasma samples and extraction eluent were supposed to suppress pH-dependent acyl migration and hydrolysis of AcMPAG. We also found that plasma acidification was indispensable for quantifying the plasma levels of AcMPAG. In contrast to present results, Patel et al. reported that AcMPAG was stable up to 24 h in nonacidified human plasma at 25 °C. This discrepancy may be

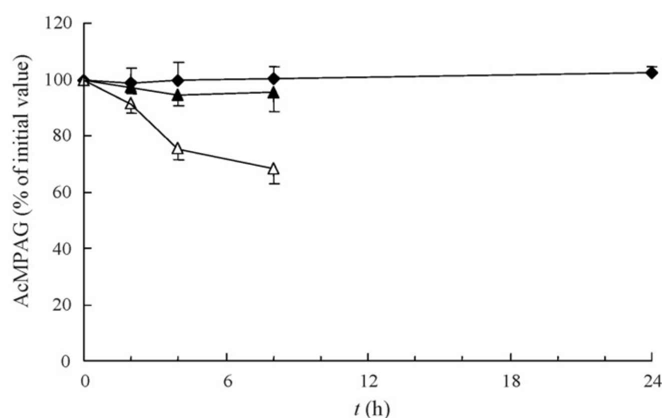

Fig. 3. Stability of AcMPAG in acidified human plasma (pH < 6.5) at 4 °C (filled squares), nonacidified plasma (pH 7.4) at 4 °C (filled triangles) and nonacidified human plasma at 20 °C (open triangles) after 2, 4, 8 and 24 h of storage. Data are expressed as means and S.D. of a percentage of the initial value ($n = 3$).

Table 2
Parameters of analytical performance of the HPLC method for MPA and its glucuronides

Sample analytes	Theoretical value (μg/ml)	Intra-assay (n = 8)			Inter-assay (n = 8)		
		Mean ± S.D. (μg/ml)	Accuracy (%)	R.S.D. (%)	Mean ± S.D. (μg/ml)	Accuracy (%)	R.S.D. (%)
MPA	0.50	0.49 ± 0.02	97.3	3.89	0.47 ± 0.03	94.3	6.45
	1.50	1.45 ± 0.02	96.8	1.41	1.49 ± 0.05	99.4	3.43
	4.00	4.09 ± 0.03	102.2	0.82	4.06 ± 0.10	101.5	2.46
	10.00	9.97 ± 0.01	99.7	0.11	9.98 ± 0.03	99.8	0.32
MPAG	20.0	19.0 ± 1.0	95.1	5.34	19.3 ± 0.6	96.5	2.92
	40.0	39.9 ± 1.2	99.8	2.98	40.8 ± 1.2	102.1	2.83
	90.0	91.0 ± 2.4	101.2	2.63	90.0 ± 1.9	100.0	2.13
	180.0	179.3 ± 0.9	99.6	0.48	179.9 ± 0.7	100.0	0.40
AcMPAG	0.25	0.25 ± 0.01	100.2	3.82	0.26 ± 0.02	105.6	6.07
	0.50	0.50 ± 0.01	99.3	2.43	0.50 ± 0.02	100.5	3.13
	1.00	1.00 ± 0.02	100.5	2.20	0.97 ± 0.04	97.1	3.77
	2.00	2.00 ± 0.01	99.9	0.42	2.01 ± 0.01	100.6	0.71

MPA, Mycophenolic acid; MPAG, mycophenolic acid phenolic glucuronide; AcMPAG, mycophenolic acid acyl glucuronide.

due to difference of methodology such as anticoagulant reagent and plasma condition. For instance, fresh plasma samples were used in this study, while Patel et al. used frozen plasma samples.

There have been 4 reports on simultaneous quantification of MPA, MPAG and AcMPAG with HPLC-UV in human plasma [20–23]. Shipkova et al. [20] and Patel and Akhlaghi [21] separated these analytes using more complicated gradient elution than isocratic elution. Using the procedure of Shipkova et al., Brandhorst et al. reported that AcMPAG could not be quantified in approximately 26% of samples because of interference [24]. Patel and Akhlaghi reported that several isocratic mobile phase compositions failed to elute MPA, MPAG and AcMPAG with acceptable resolution, especially between the peaks of AcMPAG and MPAG [21]. With isocratic elution, Khoschsorur and Erwa reported an HPLC-UV method with a mobile phase consisting of 32% acetonitrile and a 40 mM phosphoric acid buffer at pH 3.0, although plasma extracts interfered with the peaks of MPA and MPAG [22]. Westley et al. also reported an isocratic method with a mobile phase of 20% acetonitrile and 0.05% phosphoric acid for monitoring MPA, MPAG and AcMPAG. However, they were unable to quantify AcMPAG in clinical human plasma samples, most likely due to degradation of AcMPAG during storage [23]. There is also a report on quantification of AcMPAG in human plasma with LC–MS/MS [24]. With the method, the lower LOQ were 0.1 μg/ml for MPA, 1.0 μg/ml for MPAG and 0.05 μg/ml for AcMPAG, respectively. Our method is as sensitive as the MS detection and seems alternative to costly LC–MS/MS.

Plasma concentrations of AcMPAG showed interindividual variability in the early period after renal transplantation (Fig. 4). Renal function and genetic factors may also have contributed to the large interindividual variation of AcMPAG pharmacokinetics in this study. Akhlaghi et al. reported a negative correlation between the AcMPAG area under the concentration–time profile curve and glomerular filtration rate [26]. Picard et al. reported that the main enzyme involved in the formation of AcMPAG was UGT2B7 [27]. In a Japanese population, 21 single nucleotide polymorphisms of the *UGT2B7* gene were reported [28].

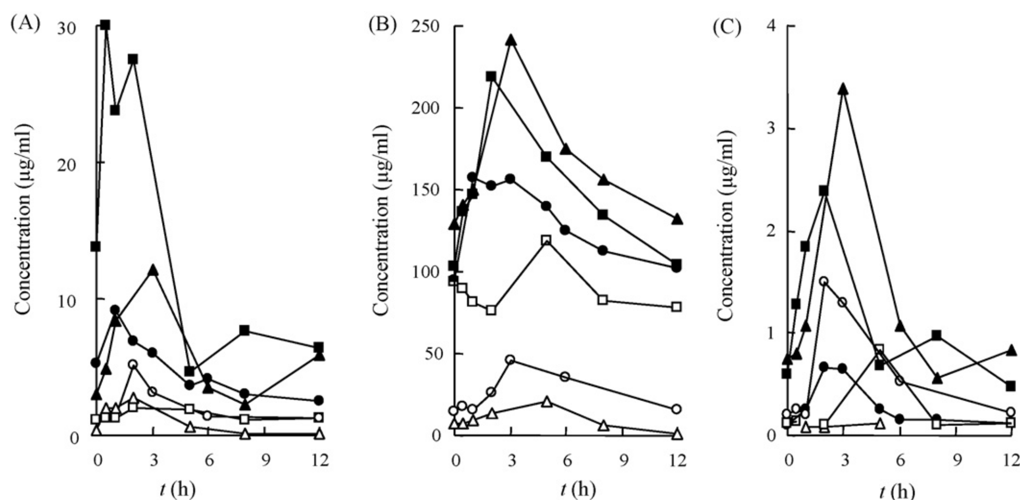

Fig. 4. Concentration–time profiles of MPA (A), MPAG (B) and AcMPAG (C) over a period of 12 h after drug administration in 6 renal transplant recipients treated with immunosuppressive therapy including MMF, tacrolimus and glucocorticoid 10 days after transplantation. Patients 1 (●), 2 (▲) and 3 (■) were on 1000 mg of MMF twice daily and patients 4 (○), 5 (△) and 6 (□) were on 500 mg of MMF twice daily.

5. Conclusion

An isocratic IP HPLC method for the simultaneous determination of MPA, MPAG and AcMPAG in human plasma has been established. The presented method is simple, accurate, reproducible and suitable for clinical application. AcMPAG is unstable in human plasma at 20 °C, and plasma acidification is necessary for quantification of AcMPAG in human plasma. AcMPAG pharmacokinetics showed a large interindividual variability as well as MPAG in the early period after renal transplantation. The method will be useful in TDM and pharmacokinetic studies of MPA and its glucuronides.

References

[1] H. Jeong, B. Kaplan, Clin. J. Am. Soc. Nephrol. 2 (2007) 184–191.

[2] B.A. Kiberd, J. Lawen, A.D. Fraser, T. Keough-Ryan, P. Belitsky, Am. J. Transplant. 4 (2004) 1079–1083.

[3] K. Cransberg, E.A. Marlies Cornelissen, J.C. Davin, K.J. Van Hoeck, M.R. Lilien, T. Stijnen, J. Nauta, Pediatr. Transplant. 9 (2005) 104–111.

[4] E.M. Eugui, S.J. Almquist, C.D. Muller, A.C. Allison, Scand. J. Immunol. 33 (1991) 161–173.

[5] M. Shipkova, V.W. Armstrong, E. Wieland, P.D. Niedmann, E. Schutz, G. Brenner-Weiss, M. Voihsel, F. Braun, M. Oellerich, Br. J. Pharmacol. 126 (1999) 1075–1082.

[6] I. Nowak, L.M. Shaw, Ther. Drug Monit. 19 (1997) 358–360.

[7] R.E. Bullingham, A.J. Nicholls, B.R. Kamm, Clin. Pharmacokinet. 34 (1998) 429–455.

[8] I. Nowak, L.M. Shaw, Clin. Chem. 41 (1995) 1011–1017.

[9] E. Schütz, M. Shipkova, V.W. Armstrong, E. Wieland, M. Oellerich, Clin. Chem. 45 (1999) 419–422.

[10] E. Wieland, M. Shipkova, U. Schellhaas, E. Schutz, P.D. Niedmann, V.W. Armstrong, M. Oellerich, Clin. Biochem. 33 (2000) 107–113.

[11] M. Shipkova, E. Wieland, E. Schutz, C. Wiese, P.D. Niedmann, M. Oellerich, V.W. Armstrong, Transplant. Proc. 33 (2001) 1080–1081.

[12] B.D. Maes, I. Dalle, K. Geboes, M. Oellerich, V.W. Armstrong, P. Evenepoel, B. Geypens, D. Kuypers, M. Shipkova, K. Geboes, Y.F. Vanrenterghem, Transplantation 75 (2003) 665–672.

[13] T. Heller, T. van Gelder, K. Budde, J.W. de Fijter, D. Kuypers, W. Arns, J. Schmidt, L. Rostaing, S.H. Powis, K. Claesson, I.A. Macphee, E. Pohanka, J. Engelmayer, G. Brandhorst, M. Oellerich, V.W. Armstrong, Am. J. Transplant. 7 (2007) 1822–1831.

[14] T. van Gelder, Y. Le Meur, L.M. Shaw, M. Oellerich, D. DeNofrio, C. Holt, D.W. Holt, B. Kaplan, D. Kuypers, B. Meiser, B. Toenshoff, R.D. Mamelok, Ther. Drug Monit. 28 (2006) 145–154.

[15] V.C. Cox, M.H. Ensom, Ther. Drug Monit. 25 (2003) 137–157.

[16] H. Hosotsubo, S. Takahara, R. Imamura, M. Kyakuno, T. Tanaka, K. Yazawa, T. Hanafusa, K. Matsumiya, N. Nonomura, A. Okuyama, H. Sugimoto, Ther. Drug Monit. 23 (2001) 669–674.

[17] I.S. Westley, J.E. Ray, R.G. Morris, Ther. Drug Monit. 28 (2006) 632–636.

[18] A. Premaud, A. Rousseau, N. Picard, P. Marquet, Ther. Drug Monit. 28 (2006) 274–277.

[19] E. Schutz, M. Shipkova, V.W. Armstrong, P.D. Niedmann, L. Weber, B. Tonshoff, K. Pethig, T. Wahlers, F. Braun, B. Ringe, M. Oellerich, Transplant. Proc. 30 (1998) 1185–1187.

[20] M. Shipkova, E. Schütz, V.W. Armstrong, P.D. Niedmann, M. Oellerich, E. Wieland, Clin. Chem. 46 (2000) 365–372.

[21] C.G. Patel, F. Akhlaghi, Ther. Drug Monit. 28 (2006) 116–122.

[22] G. Khoschsorur, W. Erwa, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 799 (2004) 355–360.

[23] I.S. Westley, B.C. Sallustio, R.G. Morris, Clin. Biochem. 38 (2005) 824–829.

[24] G. Brandhorst, F. Streit, S. Goetze, M. Oellerich, V.W. Armstrong, Clin. Chem. 52 (2006) 1962–1964.

[25] Center for Drug Evaluation and Research, U.S. Food and Drug Administration, Guidance for Industry Bioanalytical Method Validation, May 22, 2001 (Available at: http://www.fda.gov/cder/guidance/4252fnl.htm and accessed on September 12, 2007).

[26] F. Akhlaghi, C.G. Patel, X.P. Zuniga, J. Halilovic, I.S. Preis, R.Y. Gohh, Ther. Drug Monit. 28 (2006) 95–101.

[27] N. Picard, D. Ratanasavanh, A. Prémaud, Y. Le Meur, P. Marquet, Drug Metab. Dispos. 33 (2005) 139–146.

[28] M. Saeki, Y. Saito, H. Jinno, T. Tanaka-Kagawa, A. Ohno, S. Ozawa, K. Ueno, S. Kamakura, N. Kamatani, K. Komamura, M. Kitakaze, J. Sawada, Drug Metab. Dispos. 32 (2004) 1048–1054.