View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Elsevier - Publisher Connector

Kidney International, Vol. 61 (2002), pp. 1894–1898

Influence of mycophenolic acid and tacrolimus on homocysteine metabolism

Mihaela C. Ignatescu, Josef Kletzmayr, Manuela Födinger, Christian Bieglmayer, Walter H. Hörl, and Gere Sunder-Plassmann

Division of Nephrology and Dialysis, Department of Medicine III, Division of Endocrinology, Department of Laboratory Medicine, University of Vienna, Wien, Austria

Influence of mycophenolic acid and tacrolimus on homocysteine metabolism.

Background. The effect of mycophenolate mofetil (MMF) on homocysteine (Hcy) metabolism is unknown.

Methods. This in vitro study examined whether mycophenolic acid or tacrolimus influences the formation of Hcy as determined by measuring the total Hcy (tHcy) concentrations in supernatants of human renal proximal tubule epithelial cells. Cells were incubated with and without vitamins (folate, vitamin B_6 and B_{12}) in the presence of low or high methionine concentrations at different mycophenolic acid (0, or 5, or 20 μg/mL) or tacrolimus (0, or 10, or 25 ng/mL) concentrations for 24, 48 or 72 hours. The concentration of tHcy in culture supernatants was measured by a fluorescence polarization immunoassay. The effect of MMF on tHcy plasma levels was also examined in 454 kidney graft recipients.

Results. Comparisons of tHcy levels in culture supernatants over time by four way ANOVA showed that methionine concentration ($P < 0.00001$), time ($P < 0.00001$), vitamins ($P = 0.002728$), and mycophenolic acid concentration ($P = 0.000095$) were all significant predictors of tHcy concentrations. This was due to significantly lower tHcy levels with using mycophenolic acid at a high concentration versus control at the 48- and 72-hour time points. By contrast, tacrolimus showed no effect in vitro. Among the kidney graft recipients, male patients on MMF therapy showed lower plasma tHcy concentrations as compared to those on azathioprine ($P = 0.03$).

Conclusion. Our study suggests a tHcy lowering effect of MMF in male transplant recipients, which improves the cardiovascular disease risk profile, whereas tacrolimus showed no effect.

Hyperhomocysteinemia shows an association with vascular disease [1]. Experimental studies have shown that homocysteine may exert harmful effects on endothelial cells. It interferes with several clotting factors, increases platelet aggregation, enhances lipid oxidation, and induces the proliferation of smooth muscle cells. However, the majority of studies used supraphysiological concentrations of homocysteine [1].

In stable kidney graft recipients, hyperhomocysteinemia [total homocysteine (tHcy) plasma concentrations >15 μmol/L] is present in approximately 50 to 60% of the patients [2, 3]. Total homocysteine plasma concentration correlates inversely with the glomerular filtration rate, and is roughly three times as high in hemodialysis patients as compared to healthy individuals. Therefore, tHcy would be expected to fall markedly after successful renal transplantation. Thus far, no study has explained why the post-transplant reduction in tHcy is far smaller than expected with respect to improved renal function [4]. One potential explanation for this observation is the influence of the immunosuppressive therapy on Hcy metabolism in transplant patients. Some authors reported an association of cyclosporine A therapy with elevated tHcy levels in kidney or heart allograft recipients [4–7]. This finding, however, has not been confirmed by our prior studies where cyclosporine A did not have any effect on Hcy metabolism in vitro or in vivo (abstract; Sunder-Plassmann G, *J Am Soc Nephrol* 11:726A, 2000) [8]. It has been shown recently that patients on a triple therapy with steroids, tacrolimus, and mycophenolate mofetil show lower tHcy values than those on double therapy with cyclosporine A and prednisolone or tacrolimus and prednisolone (abstract; Scolari MP, *XVIII International Congress of the Transplantation Society*, 2000, p 323).

These data suggest that mycophenolate mofetil interferes with homocysteine metabolism. Therefore, the aim of our study was to assess whether mycophenolic acid, the active metabolite of mycophenolate mofetil, may play a role in the metabolism of Hcy. Human renal proximal tubule epithelial cells (huRPTEC), an important metabolic site of Hcy, were used to test this hypothesis, and the results were compared to tacrolimus. The effect of mycophenolate mofetil on tHcy plasma concentrations also was examined in 454 kidney graft recipients.

Key words: kidney transplantation, vascular disease, hyperhomocysteinemia, mycophenolate mofetil, renal proximal tubule epithelial cells, methionine, folate, vitamin B_6, vitamin B_{12}.

Received for publication August 3, 2001
and in revised form November 27, 2001
Accepted for publication December 17, 2001

© 2002 by the International Society of Nephrology

METHODS

Cell culture

Human renal proximal tubule epithelial cells (huRPTEC; provided by BioWhittaker-Clonetics, Walkersville, MD, USA) were obtained in the second passage and subcultured according to the manufacturer's specifications, in renal epithelial cell basal medium enriched with growth factors (REGM singlequots; BioWhittaker-Clonetics). After cryopreservation in the fifth passage, cells were thawed and seeded in 175 cm^2 cell culture flasks in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal calf serum (FCS) and 5 mL of penicillin-streptomycin solution (10,000 IE penicillin + 10,000 μg streptomycin/mL).

Eighty percent confluent cells were then subcultured in six-well plates using RPMI 1640 medium supplemented with 5% FCS, 5 mL of penicillin-streptomycin, 2 mL of fungizone (250 μg/mL Amphotericin B), 5 mL of L-glutamine (29.2 mg/mL), 500 μL of gentamycin (50 mg/mL) and 2500 IE of heparin (Immuno, Vienna, Austria). One set of cells was incubated with vitamin B_{12} (0.005 mg/L), folic acid (1 mg/L) and pyridoxal-HCl (1 mg/L), and one set without these vitamins. These sets were incubated in the presence of a methionine concentration of 15 mg/L or 150 mg/L and with different concentrations of mycophenolic acid (0 μg/mL, or 5 μg/mL, or 20 μg/mL; F. Hoffmann-La Roche AG, Basel, Switzerland), or tacrolimus (0 ng/mL, or 10 ng/mL, or 25 ng/mL; Fujisawa Ireland Ltd., Killorglin, Co. Kerry, Ireland), respectively. All sets were incubated for either 24, 48 or 72 hours.

A total number of six experiments were performed using one cell batch. The viability of the cells was determined using the trypan blue method and was found to be >95%. The cell culture reagents used were obtained from Life Technologies (Vienna, Austria) unless otherwise indicated.

Measurement of total homocysteine in cell supernatants

The concentration of tHcy in the supernatants was determined in duplicates using a fluorescence polarization immunoassay (IMx analyzer; Abbott Laboratories, Abbott Park, IL, USA). The lower detection limit of the assay was 0.5 μmol/L. The IMx assay revealed comparable results for tHcy concentrations as compared to the values obtained by high performance liquid chromatography (HPLC) [9]. The detailed comparison of both methods for measurement of tHcy in cell culture supernatants will be reported elsewhere.

Patient characteristics

The influence of mycophenolate mofetil on tHcy levels of kidney graft recipients was investigated in 454 patients. The mean age of the 185 female and 269 male patients was 50.7 ± 13.3 years (mean body mass index, 25.6 ± 4.3 kg/m^2; mean time since transplantation, 3.8 ± 3.2 years; mean serum creatinine concentration, 1.8 ± 1.1 mg/dL). The estimated creatinine clearance was 54.4 ± 19.9 mL/min, the mean folate plasma level was 14.7 ± 11.0 nmol/L, and the mean vitamin B_{12} plasma level was 242 ± 129 pmol/L. These patients had participated in a previous study investigating the genetic background of hyperhomocysteinemia of renal transplant patients [10]. The cross sectional study design included two groups: The first group ($N = 326$) consisted of patients on a triple therapy (cyclosporine A, prednisone and azathioprine), and the second group of 128 patients received a triple drug regimen using cyclosporine A, prednisone, and mycophenolate mofetil. All patients gave written informed consent for this study according to the Declaration of Helsinki and the Austrian Law on Gene Technology.

For the analysis of tHcy plasma concentration of patients (normal, ≤15 μmol/L), citrated blood was drawn after an overnight fast in chilled tubes and spun within 20 minutes at 4°C at 2000 × *g*. Platelet poor plasma was stored at −70°C. The concentration of tHcy in the plasma samples was determined by HPLC as described by Araki and Sako [9].

Statistics

Continuous data are given as mean ± SD. Comparisons of tHcy levels in culture supernatants over time were performed by four way ANOVA comparing mycophenolic acid (0, or 5, or 20 μg/mL) or tacrolimus (0, or 10, or 25 ng/mL), methionine (low or high), vitamins (yes or no), time of incubation (24, 48 or 72 hours), and the interaction terms for the aforementioned factors. A three way ANOVA was performed for all measurements for the time points 24, 48, and 72 hours with the factors mycophenolic acid or tacrolimus, methionine and vitamins; *t* tests were used to compare tHcy plasma levels of the users of mycophenolate mofetil versus azathioprine.

A multiple regression model was built to identify independent predictors of tHcy plasma levels. Variables included in this model were age, time since transplantation, body mass index, serum creatinine, glomerular filtration rate, gender, primary kidney disease, folate levels, B_{12} concentration, *MTHFR* 677/1298 genotypes, and the mycophenolate mofetil use yes/no term. Skewed data were transformed logarithmically to normalize the distribution.

A P value of <0.05 was considered statistically significant. All calculations were performed using Statistica for Windows (release 4.5, Stat Soft Inc., 1993).

RESULTS

Influence of mycophenolic acid on homocysteine export from huRPTEC

The mean tHcy concentration in the culture supernatants was 3.51 ± 0.27 μmol/L under standard cell cul-

Table 1. Mycophenolic acid decreases tHcy concentrations in culture supernatants of huRPTEC

MPA		Met	tHcy *μmol/L*		
μg/L	Vitamins	*mg/L*	24 h	48 h	72 h
0	wo	15	3.70 ± 0.57	5.80 ± 0.66	6.75 ± 0.75
0	w	15	3.51 ± 0.27	5.12 ± 0.34	5.71 ± 0.45
0	wo	150	4.91 ± 0.64	7.53 ± 1.29	9.69 ± 1.34
0	w	150	4.64 ± 0.87	7.26 ± 0.88	11.11 ± 2.61
5	wo	15	3.70 ± 0.43	5.28 ± 0.28	6.86 ± 1.17
5	w	15	3.50 ± 0.62	4.62 ± 0.45	5.23 ± 0.32
5	wo	150	4.89 ± 0.85	7.28 ± 1.05	9.04 ± 0.95
5	w	150	4.05 ± 0.31	6.60 ± 0.85	8.68 ± 1.18
20	wo	15	3.65 ± 0.80	5.04 ± 0.39	5.70 ± 0.40
20	w	15	3.31 ± 0.51	4.51 ± 0.31	5.01 ± 0.23
20	wo	150	4.71 ± 0.83	6.46 ± 0.44	8.78 ± 1.19
20	w	150	4.00 ± 0.26	6.30 ± 0.82	8.64 ± 1.49

Data are mean ± SD. Abbreviations are: MPA, mycophenolic acid; Met, methionine; tHcy, total homocysteine; w, with vitamins; wo, without vitamins.

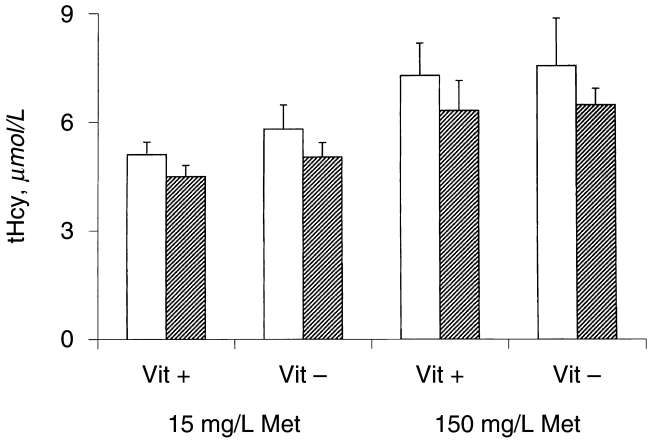

Fig. 1. Export of homocysteine (Hcy) from human renal proximal tubular epithelial cells (huRPTEC) in the presence of 20 μg/mL mycophenolic acid (▨) and under control conditions (□) with and without methionine loading and in the presence or absence of vitamins (at 48 hours). Data are mean ± SD. Abbreviations are: tHcy, total homocysteine concentration; Met, methionine; Vit +, with vitamins B_6, B_{12} and folic acid; Vit −, without vitamins B_6, B_{12} and folic acid.

ture conditions at time point 24 hours, and increased by 45.86% at 48 hours and 62.67% at 72 hours (Table 1). Comparisons of tHcy levels in culture supernatants over time by four way ANOVA showed that in vitro methionine loading ($P < 0.000001$) and prolonged incubation time ($P < 0.000001$) were associated with an increase of tHcy export from huRPTEC, whereas the presence of vitamins ($P = 0.002728$) and mycophenolic acid ($P = 0.000095$) was associated with a significant decrease in tHcy export. Separate analyses of the three different time points by three way ANOVA showed that mycophenolic acid significantly reduced tHcy export from huRPTEC at 48 and 72 hours ($P = 0.000599$ and $P = 0.0001523$, respectively; Fig. 1).

Table 2. Tacrolimus does not influence tHcy concentrations in culture supernatants of huRPTEC

Tacrolimus		Met	tHcy *μmol/L*		
ng/mL	Vitamins	*mg/L*	24 h	48 h	72 h
0	wo	15	2.05 ± 0.88	2.89 ± 0.74	3.52 ± 0.78
0	w	15	2.08 ± 0.80	3.13 ± 0.86	3.65 ± 0.76
0	wo	150	4.08 ± 1.64	5.98 ± 1.59	7.29 ± 1.43
0	w	150	4.40 ± 1.46	6.84 ± 1.68	8.28 ± 1.25
10	wo	15	1.97 ± 0.96	3.03 ± 0.96	3.42 ± 0.85
10	w	15	2.06 ± 0.93	3.08 ± 0.81	3.54 ± 0.80
10	wo	150	4.12 ± 1.26	6.00 ± 1.14	7.91 ± 1.63
10	w	150	4.41 ± 1.50	6.38 ± 1.43	7.95 ± 1.83
25	wo	15	2.15 ± 0.87	3.12 ± 0.80	3.58 ± 0.87
25	w	15	2.23 ± 0.83	3.20 ± 0.69	3.80 ± 0.77
25	wo	150	4.19 ± 1.27	6.23 ± 1.03	7.45 ± 0.87
25	w	150	4.27 ± 1.62	6.66 ± 1.64	7.83 ± 1.59

Data are mean ± SD. Abbreviations are: Met, methionine; tHcy, total homocysteine; w, with vitamins; wo, without vitamins.

Influence of tacrolimus on homocysteine export from huRPTEC

The mean tHcy concentration in the cell culture supernatants was 2.08 ± 0.8 μmol/L under standard cell culture conditions at the 24-hour time point and increased to 3.13 ± 0.86 μmol/L (50.48%) at 48 hours and to 3.65 ± 0.76 μmol/L (75.48%) at 72 hours (Table 2). Comparisons of tHcy concentrations in cell culture supernatants over time by four way ANOVA showed that methionine loading ($P < 0.000001$) and prolonged incubation time ($P < 0.000001$) were associated with an increase in tHcy export from huRPTEC. The presence of tacrolimus in the medium did not influence Hcy export ($P = 0.9733$) as compared to standard cell culture conditions. Separate analyses of the three different time points by three way ANOVA did not show an influence of tacrolimus for any of the concentrations used.

Mycophenolate mofetil use and tHcy plasma concentration in renal transplant patients

The mean tHcy plasma concentration among the entire study population ($N = 454$) was 18.2 ± 9.6 μmol/L. The mean tHcy plasma level among users of mycophenolate mofetil versus users of azathioprine was 17.5 ± 10.0 μmol/L and 18.5 ± 9.5 μmol/L, respectively. This was related to significantly lower tHcy plasma levels in male patients treated with mycophenolate mofetil versus male patients treated with azathioprine ($P = 0.030$). In the female population there was no difference between the users of azathioprine versus mycophenolate mofetil (Table 3). The independent predictors of tHcy levels are indicated in Table 4.

DISCUSSION

Our study shows that mycophenolic acid reduces homocysteine export from human renal proximal tubule epithelial cells. By contrast, tacrolimus did not have any effect on Hcy metabolism in vitro. Among 454 kidney graft

Table 3. Gender specific differences in tHcy plasma concentrations using mycophenolate mofetil versus azathioprine

Gender	MMF	AZA	*P*
Male	17.51	19.87	0.030
	N = 69	*N* = 200	
Female	17.54	16.19	0.422
	N = 59	*N* = 126	
Both	17.52	18.45	0.159

Abbreviations are: MMF, mycophenolate mofetil; AZA, azathioprine.

Table 4. Multiple regression analysis of predictors of tHcy plasma concentrations in 454 renal graft recipients

Parameter	*P*
Age	0.734
Gender	0.623
Body mass index	0.540
Time since transplantation	0.472
Body mass index	0.540
Primary kidney disease	0.881
Serum creatinine concentration	0.052
Creatinine clearance	0.118
Plasma folate concentration	0.000377
Plasma vitamin B_{12} concentration	0.00048
MTHFR 677/1298 genotypes	0.032
Use of mycophenolate mofetil	0.493

recipients, the use of mycophenolate mofetil was associated with lower tHcy plasma levels in male patients.

There is good evidence that normal kidneys play a major role in amino acid and Hcy clearance and metabolism. The existence of Hcy-metabolizing enzymes and uptake systems in renal tubular cells has been confirmed [11]. We used renal proximal tubule epithelial cells because urinary folate excretion is regulated by the degree of reabsorption of folate by the proximal tubule cells and thus is important in the metabolism of homocysteine [12]. In addition to reabsorbing folate from the tubular lumen, the proximal tubule cells secrete folate in vivo [13]. In culture, proximal tubule cells have been shown to possess folate transport pathways [14] and to maintain domain-specific nutrient transport across the epithelium [15]. Homocysteine is an endogenous sulfur-containing amino acid intermediate of the essential amino acid methionine and is not obtained from the diet. The metabolism of homocysteine is at the junction of two metabolic pathways: remethylation and transsulfuration. In remethylation, homocysteine acquires a methyl group from 5-methyltetrahydrofolate (MTHF) or from betaine to form methionine. The reaction with MTHF occurs in all tissues and is vitamin B_{12}-dependent, while the reaction with betaine is confined to the liver (and kidneys, in humans) and is vitamin B_{12}-independent [16]. Renal proximal cells take up MTHF by both the folate receptor and the reduced folate carrier, implying a role for both pathways in regulating urinary folate excretion and homocysteine metabolism [12].

The more pronounced effect of mycophenolic acid on Hcy metabolism in vitro than in vivo deserves attention. This difference may be explained by the constant and rather high concentration of mycophenolic acid used in the cell culture medium. Patients treated with mycophenolate mofetil normally receive a dose of 1 g twice daily. Looking at the pharmacokinetic of the drug, the peak plasma concentration of the active metabolite mycophenolic acid occurs roughly after two hours following ingestion. Investigation of the concentration-time profiles revealed that C_{max} was in the range of 15 to 20 μg/mL for mycophenolic acid and concentrations were <2.5 μg/mL within 12 hours after administration [17].

Another point of concern is the mechanism by which mycophenolate mofetil influences Hcy metabolism. Mycophenolic acid is the active metabolite of mycophenolate mofetil and inhibits the enzyme inosine 5′-monophosphate dehydrogenase [18]. This enzyme plays a key role in purine nucleotide biosynthesis, which is crucial for cell proliferation. The effect on lymphocyte proliferation is more pronounced because other cells are capable of using an alternate pathway for guanosine synthesis. Taken together, the effect of mycophenolic acid on tHcy plasma concentrations may be related, at least in part, to a decrease in protein turnover that is due to inhibition of cell proliferation.

Previous studies demonstrated that creatinine clearance, folate status, and *MTHFR* 677TT genotype are the most important predictors of tHcy plasma levels in kidney graft recipients [3]. The prevalence of hyperhomocysteinemia is high even in allograft recipients with normal renal function. One explanation for this observation is the potential influence of the immunosuppressive therapy on Hcy metabolism. Arnadottir et al were the first to suggest that cyclosporine A use, via a yet undefined mechanism, may be associated with higher tHcy plasma levels in kidney graft recipients [4, 5]. This association, however, was not confirmed in vitro [8] or in vivo (abstract; Sunder-Plassmann et al, *J Am Soc Nephrol* 11:726A, 2000). Of note, this US-Austrian study is the largest study on renal transplant recipients and immunosuppressive therapy to date. The present study shows that mycophenolic acid may have some tHcy plasma concentration-lowering effect in male kidney graft recipients. The most important determinants of tHcy plasma concentrations, however, remain the serum folate and vitamin B_{12} concentrations.

Following heart transplantation, an increase of tHcy plasma levels also has been observed [19]. Cole et al examined the effect of several variables on tHcy plasma concentrations in 72 heart transplant recipients. In a multiple linear regression model, only creatinine and trough cyclosporine A concentrations were independent posi-

tive predictors of tHcy [6]. Comparable results also were obtained in the study of Cook et al [7]. By contrast, azathioprine showed no influence on tHcy plasma levels in renal transplant patients [2].

In the present study, the export of Hcy by human renal proximal tubule epithelial cells was analyzed with regard to the influence of mycophenolic acid and tacrolimus, two immunosuppressive drugs often used in renal transplant recipients. Overall there was a 1.5- to twofold increase of Hcy export in the presence of methionine per day. Mycophenolic acid at high concentrations (20 μg/mL) and after an incubation time of at least 48 hours decreased Hcy export from huRPTEC. In contrast to this finding, tacrolimus did not have any influence on tHcy export at any concentration or time point. Although we did not observe an effect of tacrolimus in vitro, Quiroga et al found lower tHcy levels of tacrolimus-treated transplant patients after three and six months as compared to patients receiving cyclosporine A (abstract; Quiroga et al, *XVIII International Congress of the Transplantation Society*, 2000, p 327). In the study by Quiroga et al, significantly more patients in the tacrolimus treated group had normalized tHcy levels versus cyclosporine A treated patients after 12 months of transplantation. However, Quiroga's study did not find a significant difference in the development of cardiovascular events or histologically diagnosed chronic graft nephropathy between tacrolimus and cyclosporine A treated patients at the relatively early time point of 12 months post-transplantation.

There are some limitations in comparing observations from the cell culture model with clinical data. Although the huRPTEC is an important site of Hcy metabolism, it is not the only one. While cell culture supernatants were recovered after 24, 48, 72 hours, we couldn't provide a comparable setup to the in vivo study where plasma was withdrawn from the patients roughly 12 hours after drug ingestion. Furthermore, the effect of tacrolimus on plasma tHcy in kidney graft recipients was not examined because only a few of our patients received tacrolimus immunosuppression.

In summary, our study suggests that mycophenolic acid plays a positive role in the homocysteine metabolism by decreasing Hcy export in vitro. In vivo, male renal transplant recipients treated with mycophenolate mofetil show lower tHcy plasma levels as compared to those receiving azathioprine. Thus, mycophenolate mofetil may improve the cardiovascular disease risk profile in renal transplant recipients.

ACKNOWLEDGMENTS

The article was presented in abstract form at the 2001 annual meeting of the American Society of Transplant Physicians (ASTP) in Chicago, and at the 2001 annual meeting of the European Dialysis and Transplantation Association (EDTA) in Vienna.

Reprint requests to Mihaela Cristiana Ignatescu, M.D., Klinische Abteilung für Nephrologie und Dialyse Universitätsklinik für Innere Medizin III, Währinger Gürtel 18-20, A-1090 Wien, Austria.
E-mail: michaela.ignatescu@nephro.imed3.akh-wien.ac.at

REFERENCES

1. Sunder-Plassmann G, Winkelmayer WC, Födinger M: Therapeutic potential of total homocysteine-lowering drugs on cardiovascular disease. *Exp Opin Invest Drugs* 9:2637–2651, 2000
2. Bostom AG, Gohh RY, Tsai MY, *et al*: Excess prevalence of fasting and postmethionine-loading hyperhomocysteinemia in stable renal transplant recipients. *Arterioscler Thromb Vasc Biol* 17:1 894–1900, 1997
3. Födinger M, Wölfl G, Fischer G, *et al*: Effect of *MTHFR* 677C>T on plasma total homocysteine levels in renal graft recipients. *Kidney Int* 55:1072–1080, 1999
4. Arnadottir M, Hultberg B, Wahlberg J, *et al*: Serum total homocysteine concentration before and after renal transplantation. *Kidney Int* 54:1380–1384, 1998
5. Arnadottir M, Hultberg B, Vladov V, *et al*: Hyperhomocysteinemia in cyclosporine-treated renal transplant recipients. *Transplantation* 61:509–512, 1996
6. Cole DE, Ross HJ, Evrovski J, *et al*: Correlation between total homocysteine and cyclosporine concentrations in cardiac transplant recipients. *Clin Chem* 44:2307–2312, 1998
7. Cook RC, Tupper JK, Parker S, *et al*: Effect of immunosuppressive therapy, serum creatinine, and time after transplant on plasma total homocysteine in patients following heart transplantation. *J Heart Lung Transplant* 18:420–424, 1999
8. Ignatescu MC, Födinger M, Kletzmayr J, *et al*: The role of cyclosporine A on total homocysteine export from human renal proximal tubular epithelial cells. *Kidney Int* 59(Suppl 78):S258–S261, 2001
9. Araki A, Sako Y: Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection. *J Chromatogr* 422:43–52, 1987
10. Födinger M, Buchmayer H, Heinz G, *et al*: Effect of *MTHFR* 1298A→C and *MTHFR* 677C→T genotypes on total homocysteine, folate, and vitamin B_{12} plasma concentrations in kidney graft recipients. *J Am Soc Nephrol* 11:1918–1925, 2000
11. Friedman AN, Bostom AG, Selhub J, *et al*: The kidney and homocysteine metabolism. *J Am Soc Nephrol* 12:2181–2189, 2001
12. Sikka PK, McMartin KE: Determination of folate transport pathways in cultured rat proximal tubule cells. *Chem Biol Interact* 114: 15–31, 1998
13. Eisenga BH, McMartin KE: The effect of acute ethanol administration on the clearance of ^{3}H-PteGlu by the rat kidney. *Nutr Res* 7:1051–1060, 1987
14. McMartin KE, Morshed KM, Hazen-Martin DJ, *et al*: Folate transport and binding by cultured human proximal tubule cells. *Am J Physiol* 263:F841–F848, 1992
15. Middleton JP, Dunham CB, Onorato JJ, *et al*: Protein kinase A, cytosolic calcium, and phosphate uptake in human proximal renal cells. *Am J Physiol* 257:F631–F638, 1989
16. Bostom AG, Lathrop L: Hyperhomocysteinemia in end-stage renal disease: Prevalence, etiology, and potential relationship to arteriosclerotic outcomes. *Kidney Int* 52:10–20, 1997
17. Bullingham RES, Nicholls AJ, Kamm BR: Clinical pharmacokinetics of mycophenolate mofetil. *Clin Pharmacokinet* 34:429–455, 1998
18. Sintchak MD, Nimmesgern E: The structure of inosine 5′-monophosphate dehydrogenase and the design of novel inhibitors. *Immunopharmacol* 47:163–184, 2000
19. Berger PB, Jones JD, Olson LJ, *et al*: Increase in total plasma homocysteine concentration after cardiac transplantation. *Mayo Clin Proc* 70:125–131, 1995